AGRICULTURAL RESEARCH INSTITUTE

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# JOURNAL

## OF THE

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[1] Died January 7, 1922

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THE ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS

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Harvey W. Wiley

# PROCEEDINGS OF THE THIRTY-SIXTH ANNUAL CONVENTION OF THE ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS, 1920.

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## OFFICERS, COMMITTEES, REFEREES, AND ASSOCIATE REFEREES OF THE ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS, FOR THE YEAR ENDING OCTOBER, 1921.

*Honorary President.*

H. W. WILEY, Woodward Building, Washington, D. C.

*President*

W. F. HAND, Agricultural College, Agricultural College, Miss.

*Vice-President.*

F. P. VEITCH, Bureau of Chemistry, Washington, D. C.

*Secretary-Treasurer*

R. W. BALCOM, Box 290, Pennsylvania Avenue Station, Washington, D. C.

*Additional Members of the Executive Committee.*

A. J. PATTEN, Agricultural Experiment Station, E. Lansing, Mich.
H. D. HASKINS, Agricultural Experiment Station, Amherst, Mass.

PERMANENT COMMITTEES.

*C[illegible]tion with Other Committees on Food Definitions.*

William Frear (State College, Pa.), *Chairman.*
Julius Hortvet, St. Paul, Minn.
C. D. Howard, Concord, N. H.

*Recommendations of Referees.*

(Figures in parenthesis refer to year in which appointment expires.)

R. E. DOOLITTLE (Transportation Building, Chicago, Ill.), *Chairman.*

SUBCOMMITTEE A: B. B. Ross (1926), (Polytechnic Institute, Auburn, Ala.), *Chairman*, W. H. MacIntire (1924), C. C. McDonnell (1922). [Fertilizers (borax in fertilizers, preparation of ammonium citrate, nitrogen, potash, potash availability, precipitated phosphates), inorganic plant constituents, (sulfur and phosphorus in the seeds of plants, calcium and magnesium in the ash of seed), water, tanning materials and leather, insecticides and fungicides, and soils (sulfur in soils).]

SUBCOMMITTEE B: H. C. Lythgoe (1926), (State Department of Public Health, Boston, Mass.), *Chairman*, E. M. Bailey (1924), C. A. Browne (1922). [Foods and feeding stuffs (crude fiber, stock feed adulteration), saccharine products (honey, maple products, maltose products, sugar-house products), dairy products (moisture in cheese, cryoscopic examination of milk, methods for fat in malted milk and dried milk), fats and oils, baking powder (fluorides in baking powder), drugs (examination of arsphenamine and neoarsphenamine; determination of alcohol in drug preparations; determination of chloroform in drug preparations; determination of chloral hydrate in drug preparations; analytical methods for the determination of silver in silver proteinates; determination of camphor in pills and tablets by the alcohol distillation method; distillation method for the estimation of santalol in santal oil; turpentine; crude drugs; alkaloids; methods of analysis of morphine, codeine and diacetylmorphine; laxative and bitter tonic drugs; the determination of calomel, mercuric chloride and mercuric iodide in tablets; the analysis of acetylsalicylic acid; methods for the examination of phenolphthalein; method for the analysis of monobromated camphor; methods for the examination of procaine; preliminary report upon methods for the separation and estimation of the principal cinchona alkaloids; differentiation of Japanese and American peppermint oils), testing chemical reagents, non-alcoholic beverages, and eggs and egg products.]

SUBCOMMITTEE C: R. E. Doolittle (1926), (Transportation Building, Chicago, Ill.), *Chairman*, W. C. Geagley (1924), W. W. Randall (1922). [Food preservatives (saccharin), coloring matters (oil-soluble colors), metals in foods (arsenic), pectin in fruits and fruit products, moisture in dried fruit, canned foods, cereal foods, limit of accuracy in the determination of small amounts of alcohol in beers, vinegars, flavoring extracts, meat and meat products (separation of meat proteins, decomposition of meat products, gelatin), spices, determination of shells in cacao products, methods for the examination of cacao butter, coffee, tea, and nitrogen in foods.]

*Board of Editors.*

R. W. Balcom (Box 290, Pennsylvania Avenue Station, Washington, D. C.), *Chairman.*

E. F. Ladd (1921). R. E. Doolittle (1923).
C. B. Lipman (1922). William Frear (1924).
N. A. Parkinson, *Associate Editor.*

*Editing Methods of Analysis.*

R. E. Doolittle (Transportation Building, Chicago, Ill.), *Chairman.*

B. B. Ross. J. W. Sale.
A. J. Patten. G. W. Hoover.
W. H. MacIntire.

## Special Committees.

*Vegetation Tests on the Availability of Phosphoric Acid in Basic Slag.*

H. D. Haskins (Agricultural Experiment Station, Amherst, Mass.), *Chairman.*
C. B. Williams. B. L. Hartwell.
W. B. Ellett. J. A. Bizzell.

*Committee to Cooperate with the American Society for Testing Materials on the Subject of Agricultural Lime.*

W. H. MacIntire (Agricultural Experiment Station, Knoxville, Tenn.), *Chairman.*
William Frear. F. P. Veitch.

*Committee on Revision of Methods of Soil Analysis.*

C. B. Lipman (Agricultural Experiment Station, Berkeley, Calif.), *Chairman.*
W. H. MacIntire. R. Stewart.
A. W. Blair. J. A. Bizzell.

*Committee on Quartz-Plate Standardization and Normal Weight*

Frederick Bates (Bureau of Standards, Washington, D. C.), *Chairman.*
C. A. Browne. F. W. Zerban.

*Representative to Cooperate with the Revision Committee of the United States Pharmacopeia.*

L. F. Kebler, Bureau of Chemistry, Washington, D. C.

*Representatives on the Board of Governors of the Crop Protection Institute of the National Research Council.*

B. L. Hartwell, Kingston, R. I.
H. J. Patterson, College Park, Md.

*Referees and Associate Referees.*

*Fertilizers:*

*Referee:* R. N. Brackett, Clemson Agricultural College, Clemson College, S. C.

*Borax in fertilizers:*

*Associate referee:* W. H. Ross, Bureau of Plant Industry, Washington, D. C.

*Preparation of ammonium citrate:*

*Associate referee:* C. S. Robinson, Agricultural Experiment Station, E. Lansing, Mich.

*Nitrogen:*

*Associate referee:* I. K. Phelps, Bureau of Chemistry, Washington, D. C.

*Potash:*

*Associate referee:* J. T. Foy, Clemson Agricultural College, Clemson College, S. C.

*Potash availability:*

*Associate referee:* A. G. McCall, Agricultural Experiment Station, College Park, Md.

*Precipitated phosphates:*

*Associate referee:* H. D. Haskins, Agricultural Experiment Station, Amherst, Mass.

*Inorganic plant constituents:*

*Referee:* A. J. Patten, Agricultural Experiment Station, E. Lansing, Mich.

*Sulfur and phosphorus in the seeds of plants:*

*Associate referee:* W. L. Latshaw, Agricultural Experiment Station, Manhattan, Kans.

*Calcium and magnesium in the ash of seed:*

*Associate referee:* A. J. Patten, Agricultural Experiment Station, E. Lansing, Mich.

*Water:*

*Referee:* J. W. Sale, Bureau of Chemistry, Washington, D. C.

*Tanning materials and leather:*

*Referee:* F. P. Veitch, Bureau of Chemistry, Washington, D. C.

*Insecticides and fungicides:*

*Referee:* J. J. T. Graham, Bureau of Chemistry, Washington, D. C.

*Soils:*

*Referee:* W. H. MacIntire, Agricultural Experiment Station, Knoxville, Tenn.

*Sulfur in soils:*

*Associate referee:* W. H. MacIntire, Agricultural Experiment Station, Knoxville, Tenn.

*Foods and feeding stuffs:*

*Referee:* J. B. Reed, Bureau of Chemistry, Washington, D. C.

*Crude fiber:*

*Associate referee:* G. L. Bidwell, Bureau of Chemistry, Washington, D. C.

*Stock feed adulteration:*

*Associate referee:* H. E. Gensler, State Department of Agriculture, Harrisburg, Pa.

*Saccharine products:*

*Referee:* H. S. Paine, Bureau of Chemistry, Washington, D. C.

*Honey:*

*Associate referee:* S. F. Sherwood, Bureau of Plant Industry, Washington, D. C.

*Maple products:*

*Associate referee:* C. H. Jones, Agricultural Experiment Station, Burlington, Vt.

*Maltose products:*

*Associate referee:* O. S. Keener, Bureau of Chemistry, Washington, D. C.

*Sugar-house products:*

*Associate referee:* J. F. Brewster, Sugar Station, New Orleans, La.

*Dairy products:*

*Referee:* Julius Hortvet, State Dairy and Food Commission, St. Paul, Minn.

*Moisture in cheese:*

*Associate referee:* L. C. Mitchell, U. S. Food and Drug Inspection Station 310 Federal Office Building, Minneapolis, Minn.

*Cryoscopic examination of milk:*

*Associate referee:* E. M. Bailey, Agricultural Experiment Station, New Haven, Conn.

*Methods for fat in malted milk and dried milk:*

*Associate referee:* J. T. Keister, Bureau of Chemistry, Washington, D. C.

*Fats and oils:*

*Referee:* G. S. Jamieson, Bureau of Chemistry, Washington, D. C.

*Baking powder:*

*Referee:* L. H. Bailey, Bureau of Chemistry, Washington, D. C.

*Fluorides in baking powder:*

*Associate referee:* J. K. Morton, Bureau of Chemistry, Washington, D. C.

*Drugs:*

*Referee:* G. W. Hoover, U. S. Food and Drug Inspection Station, Transportation Building, Chicago, Ill.

*Determination of alcohol in drug preparations:*

*Associate referee:* A. G. Murray, Bureau of Chemistry, Washington, D. C.

*Determination of chloroform in drug preparations:*

*Associate referee:* A. G. Murray, Bureau of Chemistry, Washington, D. C.

*Determination of chloral hydrate in drug preparations:*

*Associate referee:* A. G. Murray, Bureau of Chemistry, Washington, D. C.

*Analytical methods for the determination of silver in silver proteinates:*

*Associate referee:* W. L. Mitchell, Room 1012, U. S. Appraiser's Stores, New York, N. Y.

*Determination of camphor in pills and tablets by the alcohol distillation method:*

*Associate referee:* G. H. Arner, Room 1012, U. S. Appraiser's Stores, New York, N. Y.

*Distillation method for the estimation of santalol in santal oil:*

*Associate referee:* C. W. Harrison, U. S. Food and Drug Inspection Station, Park Avenue Building, Baltimore, Md.

*Turpentine:*

*Associate referee:* J. O. Clarke, U. S. Food and Drug Inspection Station, U. S. Custom House, Savannah, Ga.

*Crude drugs:*

*Associate referee:* Arno Viehoever, Bureau of Chemistry, Washington, D. C.

*Alkaloids:*

*Associate referee:* A. R. Bliss, Emory University, Emory University, Ga.

*Methods for analysis of morphine, codeine and diacetylmorphine:*

*Associate referee:* C. K. Glycart, U. S. Food and Drug Inspection Station, Transportation Building, Chicago, Ill.

*Laxative and bitter tonic drugs:*

*Associate referee:* H. C. Fuller, Institute of Industrial Research, Washington, D. C.

*Determination of calomel, mercuric chloride and mercuric iodide in tablets:*

*Associate referee:* E. C. Merrill, United Drug Company, Boston, Mass.

*Analysis of acetyl salicylic acid:*

*Associate referee:* A. E. Paul, U. S. Food and Drug Inspection Station, Government Building, Cincinnati, Ohio.

*Methods for the examination of phenolphthalein:*

*Associate referee:* Samuel Palkin, Bureau of Chemistry, Washington, D. C.

*Methods for the analysis of monobromated camphor:*

*Associate referee:* C. D. Wright, Bureau of Chemistry, Washington, D. C.

*Methods for the examination of procaine:*

*Associate referee:* A. W. Hanson, U. S. Food and Drug Inspection Station, Transportation Building, Chicago, Ill.

*Methods for the separation and estimation of the principal cinchona alkaloids:*

*Associate referee:* E. O. Eaton, U. S. Food and Drug Inspection Station, U. S. Appraiser's Stores, San Francisco, Calif.

*Differentiation of Japanese and American peppermint oils:*

*Associate referee:* E. O. Eaton, U. S. Food and Drug Inspection Station, U. S. Appraiser's Stores, San Francisco, Calif.

*Testing chemical reagents:*

*Referee:* G. C. Spencer, Bureau of Chemistry, Washington, D. C.

*Non-alcoholic beverages:*

*Referee:* W. W. Skinner, Bureau of Chemistry, Washington, D. C.

*Eggs and egg products:*

*Referee:* H. L. Lourie, U. S. Food and Drug Inspection Station, U. S. Appraiser's Stores, New York, N. Y.

*Food preservatives (saccharin):*

*Referee:* M. G. Wolfe, U. S. Food and Drug Inspection Station, U. S. Appraiser's Stores, New York, N. Y.

*Coloring matters (oil-soluble colors):*

*Referee:* W. E. Mathewson, Bureau of Chemistry, Washington, D. C.

*Metals in foods:*

*Referee:* W. F. Clarke, Bureau of Chemistry, Washington, D. C.

*Arsenic:*

*Associate referee:* R. M. Hann, Bureau of Chemistry, Washington, D. C.

*Pectin in fruits and fruit products:*

*Referee:* H. J. Wichmann, U. S. Food and Drug Inspection Station, Tabor Opera House Building, Denver, Colo.

*Moisture in dried fruit:*

*Referee:* R. W. Hilts, U. S. Food and Drug Inspection Station, U. S. Appraiser's Stores, San Francisco, Calif.

*Canned foods:*

*Referee:* R. W. Balcom, Bureau of Chemistry, Washington, D. C.

*Cereal foods:*

*Referee:* C. H. Bailey, Agricultural Experiment Station, University Farm, St. Paul, Minn.

*Limit of accuracy in the determination of small amounts of alcohol in beers:*

*Referee:* J. R. Eoff, 4104 N. Union Ave., St. Louis, Mo.

*Vinegars:*

*Referee:* W. C. Geagley, State Dairy and Food Department, Lansing, Mich.

*Flavoring extracts:*

*Referee:* W. W. Skinner, Bureau of Chemistry, Washington, D. C.

*Meat and meat products:*

*Referee:* C. R. Moulton, University of Missouri, Columbia, Mo.

*Separation of meat proteins:*

*Associate referee:* C. R. Moulton, University of Missouri, Columbia, Mo.

*Decomposition of meat products:*

*Associate referee:* (Not appointed.)

*Gelatin:*

*Associate referee:* C. R. Smith, Bureau of Chemistry, Washington, D. C.

*Spices:*

*Referee:* A. E. Paul, U. S. Food and Drug Inspection Station, Transportation Building, Chicago, Ill.

*Determination of shells in cacao products:*

*Referee:* B. H. Silberberg, Bureau of Chemistry, Washington, D. C.

*Methods for the examination of cacao butter:*

*Referee:* W. F. Baughman, Bureau of Chemistry, Washington, D. C.

*Coffee:*

*Referee:* H. A. Lepper, Bureau of Chemistry, Washington, D. C.

*Tea:*

*Referee:* R. E. Andrew, Agricultural Experiment Station, New Haven, Conn.

*Nitrogen in foods:*

*Referee:* I. K. Phelps, Bureau of Chemistry, Washington, D. C.

## MEMBERS AND VISITORS PRESENT, 1920 MEETING.

Abbott, J. S., Institute of Independent Manufacturers of Margarine, 1212 Munsey Building, Washington, D. C.
Albrech, M. C., The R. T. French Company, Rochester, N. Y.
Alexander, Miss L. M., Bureau of Markets, Washington, D. C.
Allen, W. M., State Department of Agriculture, Raleigh, N. C.
Alsberg, C. L., Food Research Institute, Stanford University, Calif.
Ames, J. W., Agricultural Experiment Station, Wooster, Ohio.
Anderson, M. S., Bureau of Soils, Washington, D. C.

Badger, C. H., Bureau of Chemistry, Washington, D. C.
Bailey, C. H., Agricultural Experiment Station, University Farm, St. Paul, Minn.
Bailey, E. M., Agricultural Experiment Station, New Haven, Conn.
Bailey, L. H., Bureau of Chemistry, Washington, D. C.
Balch, R. T., Bureau of Chemistry, Washington, D. C.
Balcom, R. W., Bureau of Chemistry, Washington, D. C.
Ball, C. O., National Canners Association, 1739 H Street, N. W., Washington, D. C.
Barnard, H. E., American Institute of Baking, Minneapolis, Minn.
Bartlett, G. M., Joseph Campbell Co., Camden, N. J.
Bartlett, J. M., Agricultural Experiment Station, Orono, Me.
Bates, Carleton, U. S. Glue Company, Milwaukee, Wis.
Bates, Frederick, Bureau of Standards, Washington, D. C.
Baughman, W. F., Bureau of Chemistry, Washington, D. C.
Beyer, G. F., Bureau of Internal Revenue, Washington, D. C.
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# PRESIDENT'S ADDRESS[1].

## THE APPLICATION OF THE THEORY OF PROBABILITY TO THE INTERPRETATION OF MILK ANALYSES.

By H. C. LYTHGOE (State Department of Public Health, Boston, Mass.), *President.*

In 1914 I published an article upon the composition of milk[2]. The article resulted from the compilation of the analyses of about 500 samples of milk of known purity of which 63 represented herds and the balance represented milk from individual cows. The conclusions recorded in the article were based upon the best judgment of the results obtained and were not intended to represent the last word upon milk analysis and milk composition, neither were they intended to give to milk handlers the right to adulterate the product they sold until it conformed with the worst milk that could be produced by a cow or a small herd of cows and to use the conclusions of the article as evidence in court that the adulterated milk was pure.

The adulteration of milk with water is risky, and will eventually be detected although the profits are high and the detection of small quantities of added water is difficult and, in many instances, impossible. The work upon milk serum reported by Nurenberg in 1914[3] and 1915[4] has served as a supplement to the conclusions in the above-mentioned article upon the detection of added water, and this phase of the question needs no elucidation at present.

Adulteration by the removal of cream or, in other words, by the addition of skimmed milk is highly profitable, is difficult of detection and probably is not uncommon. Because of the demand for cream, there is a large surplus of skimmed milk left upon the dealers' hands and it is much more profitable to pass this on to the consumer as whole milk at 19 cents per quart than as skimmed milk at 5 cents per quart, particularly since the public has not shown any desire to purchase this

[1] Presented Tuesday morning, November 16, 1920, as special order of business for 11.30 o'clock.
[2] *J. Ind. Eng. Chem.*, 1914, 6: 899.
[3] *J. Assoc. Official Agr. Chemists*, 1916, 2: 8.
[4] *Ibid.*, 145; Rept. Mass. State Dept. Health, 1915, 517.

skimmed milk at the prices at which the dealers desire to sell, and this skimmed milk, therefore, is practically a waste product.

During the recent milk price-fixing regime of the Federal Milk Commission for New England, the dealers bought from the producers at a price based upon a fat content of 3.5 per cent and, presumably, the retail price was based upon the same fat content. Because a premium was paid for milk with a fat content above 3.5 per cent the producers began to ship milk containing from 3.8 to 4.0 per cent of fat. This milk was then presumably diluted with skimmed milk to bring the fat down to 3.5 to 3.7 per cent and, occasionally, to 3.3 to 3.4 per cent. To substantiate this statement, reference is made to Chart I, which shows the relation between the solids and fat of: (1) Milk of known purity (from that portion of a curve of 1000 analyses from individual cows lying between 11.5 and 13.5 per cent of solids); (2) milk from the so-called large dealers in Massachusetts taken during 1913 and 1914; and (3), milk from the same dealers from December 1917 to July 1918. It should be carefully noted that the curve representing the commercial milk of 1913-1914, parallels that of the known-purity samples but the fat is slightly lower in the commercial samples. The commercial samples of 1918 show a remarkable unanimity in fat content, irrespective of what the solids may be. This can be explained by the presence of skimmed milk in the latter samples. Since the protein-fat ratio in these samples was less than 1.0, it is evident that the interpretation of milk analyses must be different when the sample represents the combined milk of a number of dairies than when it represents the milk of but one cow or of a small dairy. It should be stated that when the results of the 1919 analyses were not compiled collectively but each dealer's milk was compiled separately, no such unanimity of results was obtained.

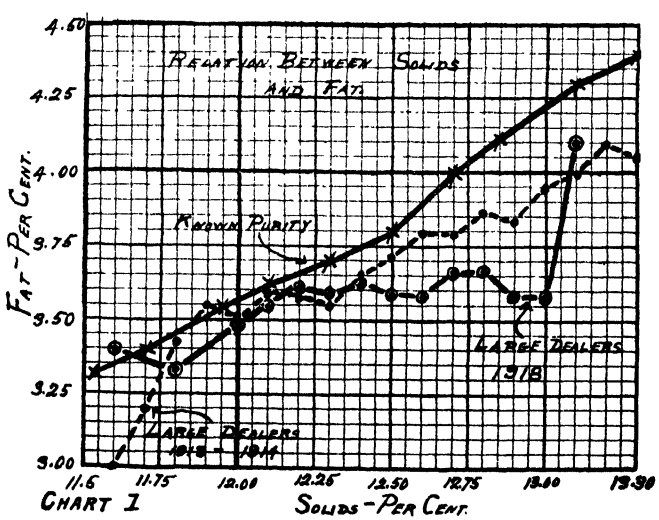



CHART I

In the conclusions of the article previously referred to, the following statements occur[1]:

"The protein-fat ratios in all cases have been less than 1. If this figure exceeds 1, skimming is indicated, the amount being greatest in samples possessing the highest ratio.

"If the protein-fat ratio is less than 0.7 or the percentage of fat in the solids is above 35.0, samples may be declared watered by a low refraction of the serum, not necessarily below the minimum for all samples of known purity. This is particularly true when dealing with herd milk."

The legal mind has attempted to misconstrue the first statement by claiming that it is capable of converse construction and means that if the protein-fat ratio is less than 1.0 the milk is pure. The falseness of this contention can be understood easily by any one of average mentality, particularly so when one realizes that the average market milk has a protein-fat ratio of 0.82 and the mixed milk of the Guernsey and Jersey type of cows has a protein-fat ratio as low as 0.6. Considerable skimmed milk, therefore, can be added to such milk before the ratio of proteins to fat reaches 1.0. Milk with a fat content of 4 per cent and a protein-fat ratio of 0.82 can be adulterated with 15 per cent of skimmed milk and still possess a protein-fat ratio of less than 1.0. It is evident, therefore, that the use of a protein-fat ratio of less than 1.0 as a criterion that milk is not skimmed is faulty and, therefore, when dealing with the composite milk of a number of herds it is reasonable to use a lower figure for the detection of skimming, particularly so if other figures point to the fact that the sample, before being tampered with, naturally had a low or average protein-fat ratio.

The second quotation is of interest in that if milk of a low protein-fat ratio naturally possesses a high refracting serum, milk with a high refracting serum naturally possesses a low protein-fat ratio. In other words, it is not usual for milk to possess both a high refraction of the serum and a high protein-fat ratio.

In the samples referred to, the copper serum refraction and the protein-fat ratio were determined upon 362 samples. The average protein-fat ratio has been computed for each 0.1 variation in the serum refraction, each computation representing from 1 to 24 samples averaging 10.4. The results are shown in Chart II. The heavy, flattened curve shows the resultant of this relation if more samples had been included. In all cases when the average protein-fat ratio was above 0.85 the average refraction was below 37. In all cases when the refraction was above 38.0 the average protein-fat ratio was below 0.81. Deductions from the flattened curve indicate that mixtures of milk from many dairies with a copper serum refraction above 38 would have a protein-fat ratio of less

[1] *J. Ind. Eng. Chem.*, 1914, 6: 907.

than 0.80 and, therefore, it may be assumed that milk representing a composite sample from many dairies, with a protein-fat ratio of 0.90 or above, has in some manner been diluted with skimmed milk, particularly so if the refractive index of the copper serum is 38 or above.

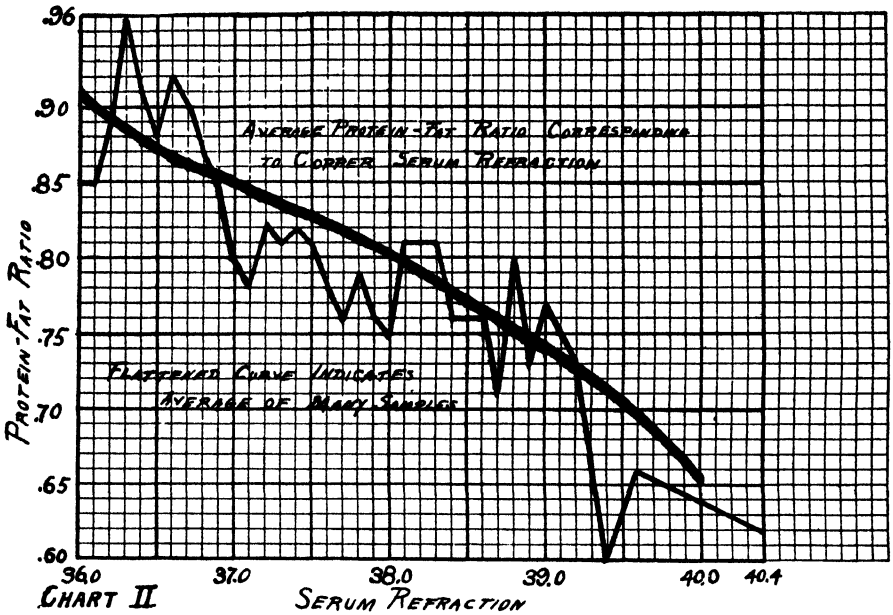



CHART II

The foregoing statements are from an article prepared in 1919 and withheld from publication in order that a more detailed study might be given to the protein-fat ratio of milk and its relations to various other milk ingredients. As first shown by Van Slyke[1], the protein-fat ratio is a characteristic of the breed and in all natural milk is less than 1.0; if it exceeds 1.0, skimming is indicated. This has been confirmed by work of the Massachusetts Department of Public Health. Other variations in the protein-fat ratio, possibly of minor character but, nevertheless, of significance, are in variations with changes in solids, fat and serum refraction, as well as variations in herd milk compared with milk from individual cows. All these variations have a bearing upon the interpretation of analyses when the possibility of skimming is to be considered and the protein-fat ratio is less than 1.0.

In order to properly compile and study these variations, the arithmetic probability paper of Hazen and Whipple[2] was employed. The ordinates of this paper may be either arithmetic or logarithmic but the abscissae constitute a probability scale of such nature that if "the items of a series of observations plotted on this paper fall in a straight line it indicates

[1] *J. Am. Chem. Soc.*, 1908, **30**: 1166.
[2] *J. Frank. Inst.*, 1916, **182**: 37, 205.

that they form a probability series; that is, they occur according to the laws of chance." This paper is of value for such observations as age of scholars, rainfall, flow of streams, death rates, bacteria counts, etc. As a rule, the arithmetic paper is preferable for most observations, but in some instances the observations plot in a curved line, as in the case of certain bacterial observations. For such observations, the logarithmic paper is preferable.

In all statistical work a large series of observations is desirable for satisfactory results, but by the use of this paper, in the case of a relatively small number of observations, it is possible to ascertain whether or not the observations are of such a nature that conclusions can be drawn from them. In other words, this paper eliminates the freaks from a series of observations. An example of the use of this paper in connection with the examination of food was shown by L. I. Nurenberg in his report to this association on dairy products in 1915.[1]

In carrying out this study, I had at my disposal the analyses of the milk of over 1000 individual cows, and of 116 herds, the samples being milked in the presence of an inspector or a chemist of the Massachusetts Department of Public Health. The cows were representations of all the usual dairy breeds and crossbreeds; they were of various ages; represented all periods of lactation, and the samples were collected at all seasons of the year.

A study made in 1919 of the herd milk figures gave the surprising information that, although the average protein-fat ratio is about 0.83, 11 per cent had a protein-fat ratio above 0.90, and except for the maximum value 0.96 and the 3 minimum values 0.55, 0.56 and 0.60, the data plotted approximately upon a straight line on the probability paper. A subdivision of these figures into milk below 12 per cent and above 13 per cent in solids showed a much larger percentage of milk with high protein-fat ratio in the case of those samples below 12 per cent than in the case of the entire number. For example, 3 per cent of the samples above 13 per cent in solids had a protein-fat ratio above 0.90, and 13 per cent of those below 12 per cent in solids were above 0.90, and of those between 12 and 12.9 per cent solids, 20 per cent were above 0.90. This compilation of results on samples below 12 per cent in solids, representing but 13 samples, does not plot in a straight line, and, therefore, definite conclusions can not be drawn from those figures; but from the similarity beween the different plots with the same general direction in all cases, it is evident that the percentage of samples with high protein-fat ratio in herd milk with solids of less than 12 per cent must necessarily greatly exceed that in herd milk above 12 per cent in solids. It appears from the figures in Chart III that it is impracticable to call commercial milk,

[1] *J. Assoc. Official Agr. Chemists*, 1916, 2: 145.

"skimmed", if relying entirely upon a protein-fat ratio between 0.90 and 0.98.

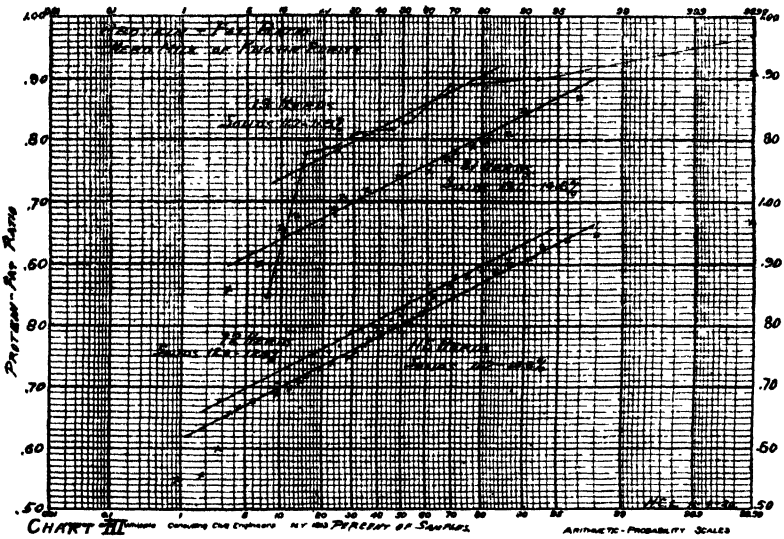



CHART III

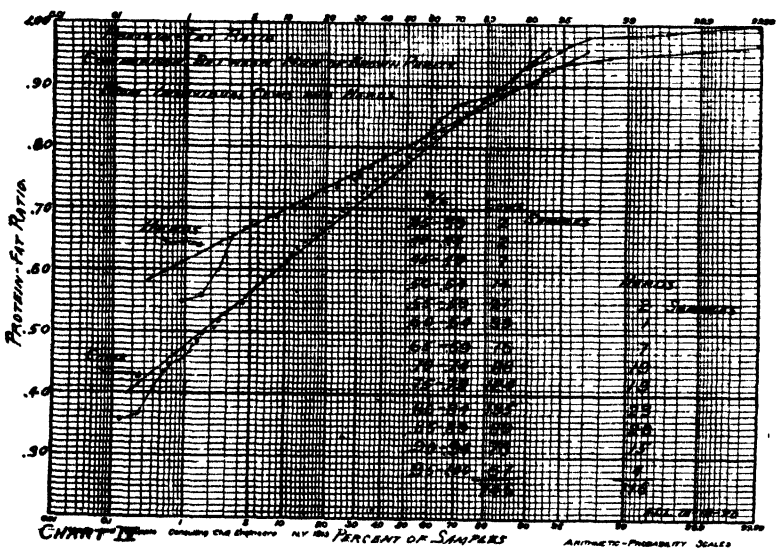
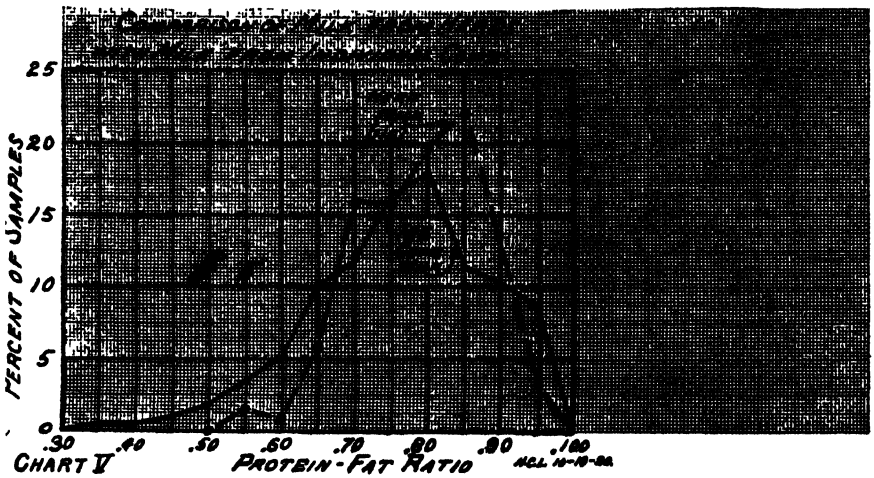


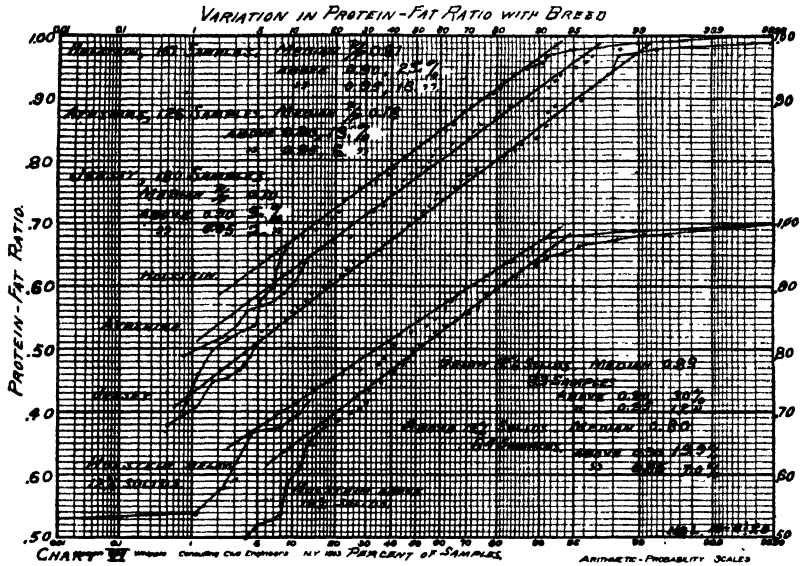

CHART IV

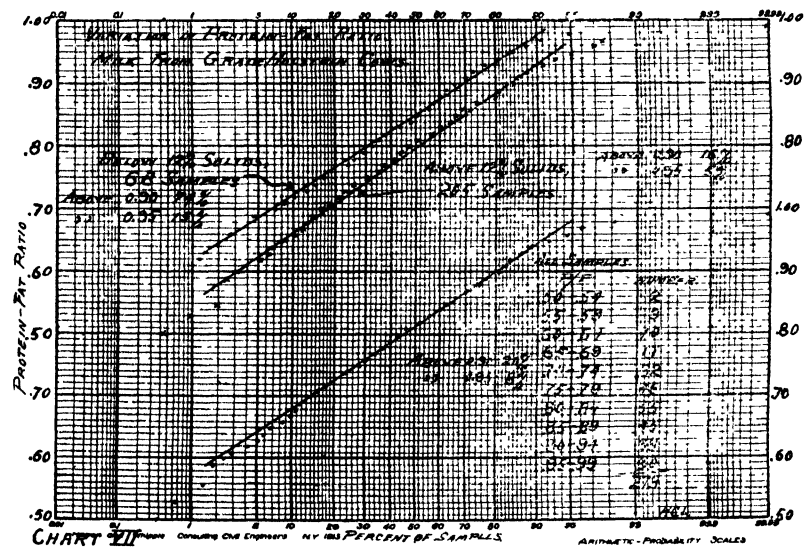



A more complete study of the protein-fat ratio was made, specifically in relation to its variation with breed, solids, fat, and copper-serum refraction, and the comparison between milk from individual cows and from herds. Chart IV (on the probability paper) and Chart V (on ordinary arithmetic paper for comparative purposes) give the comparison of milk from 746 individual cows with that from 116 herds. In the plot on the probability paper, it will be noticed that while a large number of low protein-fat ratios found in individual cows disappear in herd milk, a smaller number of the high ratios disappear in the herd milk. This is in marked contradistinction to other figures, such as solids and fat taken from analyses of the same samples, in which cases, about equal quantity of high and low figures in milk from individual cows are not to be found in herd milk. This is further emphasized by the fact that the median, which in these figures closely approximates the average, is about 0.80, while the arithmetic mean of the maximum and the minimum is about 0.70, showing a far greater preponderance of milk with a high protein-fat ratio than with a low protein-fat ratio. It is possible that the underlying cause for this condition is due to the preponderance of certain breeds of cattle.

The 746 samples from individual cows were obtained according to breeds as follows:

167 from pure-bred Holsteins;
126 from pure-bred Ayrshires and a few grades;
180 from pure-bred and grades of the Jersey and Guernsey types;
273 from grade cows of the Holstein type.




The variation in protein-fat ratio with the breed first pointed out by Van Slyke, as previously stated, is clearly demonstrated in Chart VI, the Holstein breed giving milk with the highest protein-fat ratio, and the cows of the Jersey and Guernsey types giving milk with the lowest protein-fat ratio. The milk from the grade Holstein cows is shown on Chart VII, and closely approximates that of the Holstein breed. It should be noted that the percentage of the samples above 0.90 in the different

breeds follows very closely the variation in the average protein-fat ratio of that herd. Thus, 25 per cent of the samples from the Holstein cows (average protein-fat ratio 0.87 according to Van Slyke), 13 per cent of the milk from the Ayrshire cows (average protein-fat ratio 0.82), and 5 per cent of the milk from the Jersey cows (average protein-fat ratio 0.64) were above 0.90. A comparison of the median with the arithmetic mean of the highest and lowest is of interest in these figures, giving further evidence that the prevalence of high protein-fat ratios is due to breeding, and is as follows:

*Comparison of the median with the arithmetic mean.*

| BREED | MEDIAN | ARITHMETIC MEAN OF HIGHEST AND LOWEST |
|---|---|---|
| Jersey | 0.70 | 0.685 |
| Ayrshire | 0.77 | 0.74 |
| Grade Holstein | 0.81 | 0.745 |
| Holstein | 0.82 | 0.70 |

It is evident that the question of breeds must be eliminated if figures below 0.99 are to be used in detecting skimming. Therefore, the protein-fat ratio was studied in respect to its variations with variations in other milk constituents, and it was found that the protein-fat ratio was to some extent a function of the fat, of the copper-serum refraction, and to a less extent of the solids.

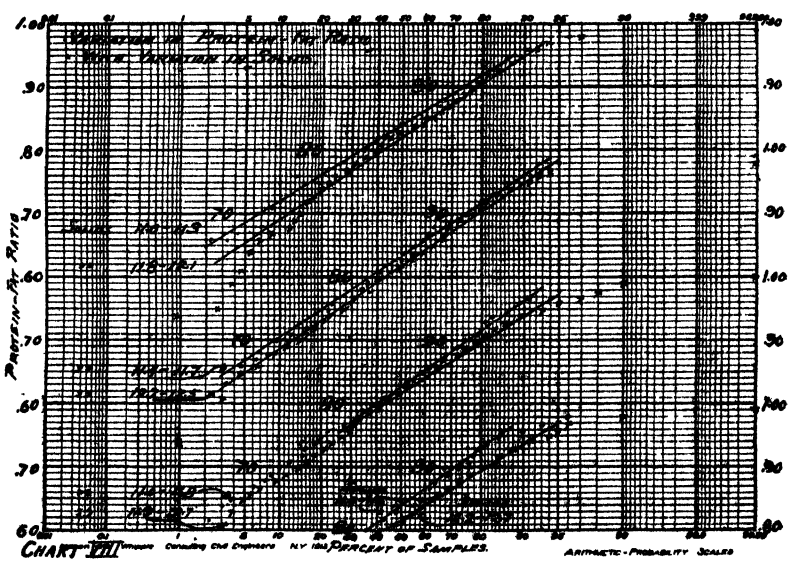
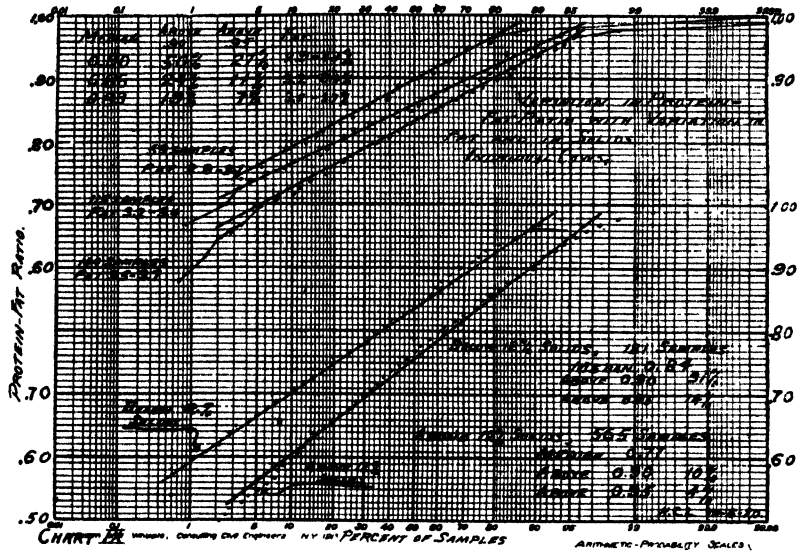
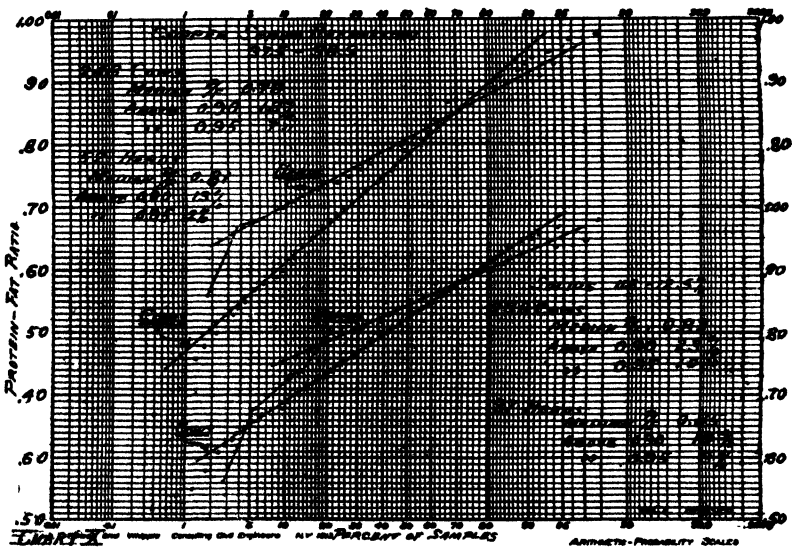



CHART VII

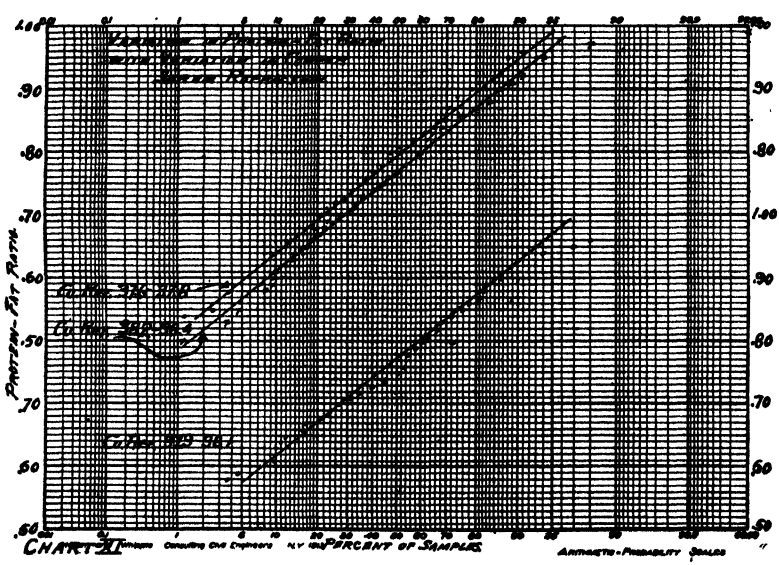


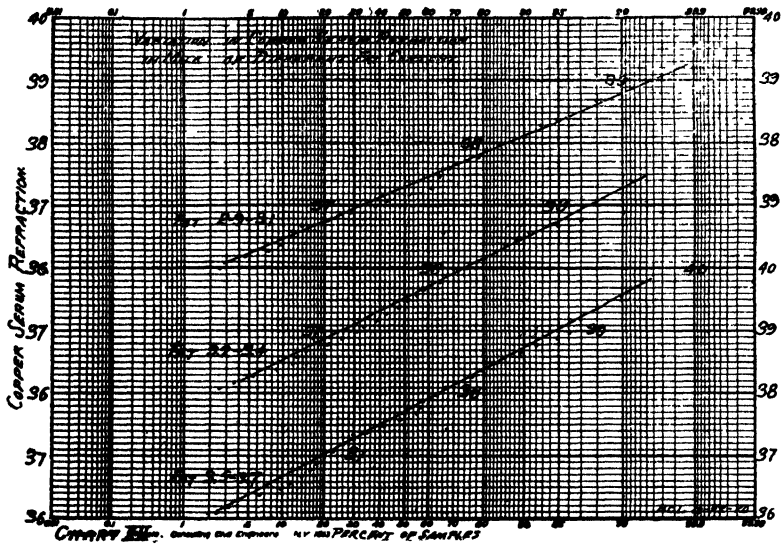




Some of the plots in Charts VI, VII, IX and X show a division of certain milk samples with groups above and below 12 per cent of solids, and it will be noted that those below 12 per cent in solids have a much greater percentage of samples with protein-fat ratio above 0.90. This is also evident from a perusal of the results of herd samples in Chart III. In Chart VIII is given the variation in protein-fat ratio with the variation in solids within narrow limits, showing that as the solids decrease

the protein-fat ratio increases. Three hundred and eighty-seven samples are represented in this chart. There were 312 samples with fat percentages between 2.9 and 3.7. They were subdivided into three groups with fat variation of 0.3 each: namely, 2.9 to 3.1 per cent; 3.2 to 3.4 per cent; and 3.5 to 3.7 per cent. The results of this compilation are shown in Chart IX. The samples with fat between 2.9 and 3.1 per cent seem to fall into a somewhat different class than the others, and about half of the samples had a protein-fat ratio above 0.90, which is the highest percentage of any of the groupings studied. Of the samples with fat between 3.2 and 3.4 per cent fat, 24 per cent had a protein-fat ratio above 0.90, and of the samples between 3.5 per cent and 3.7 per cent fat, 19 per cent had a protein-fat ratio above 0.90. It is in commercial milk with fat content between 3.2 and 3.7 per cent that the greatest difficulty is encountered in the detection of the presence of skimmed milk.

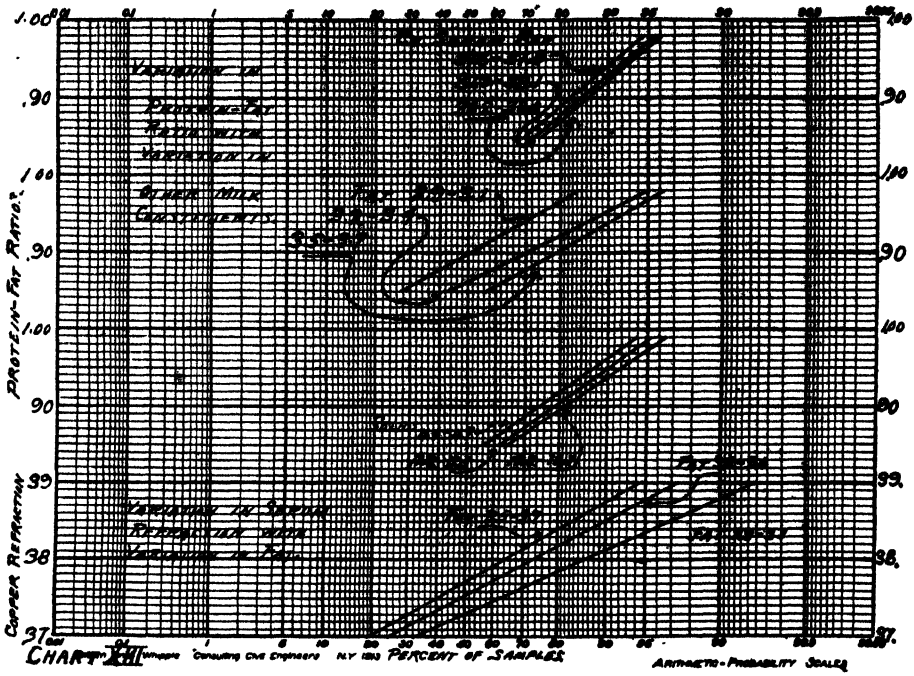
To further study the relation beween protein-fat ratio and copper-serum refraction, the protein-fat ratio of milk with copper-serum refraction between 37.5 and 38.3 from 248 cows and 52 herds was plotted and is shown in Chart X. This chart also illustrated again the preponderance of high protein-fat ratios in contradistinction to those of low protein-fat ratio, and also shows that more low and less high protein-fat ratio figures disappear in herd milk. Note, however, that 13 per cent of the herd samples, copper-serum refraction 37.5 to 38.3, were above 0.90 in protein-fat ratio, thus showing the fallibility of the conclusion in the preliminary discussion of this question, drawn entirely from a study of averages.






To study this variation in narrower ranges, Chart XI was prepared, showing the variation in protein-fat ratio with the variation in copper-serum refraction: 77 samples, copper-serum refraction 37.6 to 37.8; 82 samples, copper-serum refraction 37.9 to 38.1; 87 samples, copper-serum refraction 38.2 to 38.4. In general, these plots show that as the copper-serum refraction increases the protein-fat ratio diminishes, but in each plot it will be noticed that from 13 to 18 per cent of the samples were above 0.90 in protein-fat ratio. It may be possible to apply these data to the interpretation of analyses, but, in order to obtain another factor, the probability of variation between copper-serum refraction and fat was computed for certain fat percentages and will be found in Chart XII, showing that to some extent the serum refraction is a function of the fat percentage.

It is manifest that conclusive opinions relative to the removal of cream can not be given in the cases referred to unless they occur so extensively that the probability of their natural occurrence has been eliminated by an overwhelming number of samples of unusual composition. For comparative purposes and for ease of study, that portion of the variation of the protein-fat ratio between 0.85 and 0.99, as compared with variation of serum refraction, fat and solids, together with the variation of the serum refraction between 37 and 39, as compared with the variation in fat, will be found in Chart XIII. Since the plots were prepared largely from analyses of individual cow's milk, they should give sufficient margin of safety for conclusive opinions.




The following figures show the analyses of three samples of milk of known purity and the computed analyses resulting from assumed skimming.

*Effect of skimming on protein-fat ratio and copper serum.*

| SOLIDS | FAT | PROTEINS | PROTEIN-FAT RATIO | COPPER REFRACTION | CREAM REMOVED | FAT CORRESPONDING TO SOLIDS NOT LESS THAN |
|---|---|---|---|---|---|---|
| *per cent* | *per cent* | *per cent* | | *degrees* | *per cent* | *per cent* |
| 12.07 | 3.55 | 2.68 | 0.76 | 38.3 | None | 3.30 |
| 11.42 | 3.00 | 2.68 | 0.89 | 38.3 | 15 A | 2.90 |
| 11.22 | 2.80 | 2.68 | 0.96 | 38.3 | 21 B | 2.80 |
| 12.40 | 3.60 | 2.99 | 0.83 | 38.0 | None | 3.60 |
| 12.10 | 3.30 | 2.99 | 0.91 | 38.0 | 8 C | 3.40 |
| 11.90 | 3.10 | 2.99 | 0.96 | 38.0 | 14 D | 3.20 |
| 13.30 | 4.00 | 2.86 | 0.72 | 39.5 | None | 4.10 |
| 12.50 | 3.20 | 2.86 | 0.89 | 39.5 | 20 E | 3.60 |
| 12.30 | 3.00 | 2.86 | 0.96 | 39.5 | 25 F | 3.50 |

From Table 3 in the publication previously referred to[1], showing the

[1] *J. Ind. Eng. Chem.*, 1914, 6: 903.

expected relation between the fats and solids, samples B, E, and F are highly suspicious of being skimmed. The protein-fat ratio alone indicates nothing except suspicion in the cows of examples B, D and F. The following figures are computed from Chart XIII and show the probability expressed in per cent of these figures occurring.

*Probable ratio between proteins and fat.*

A, 15 PER CENT SKIMMED

| Probability of | | *per cent* |
|---|---|---|
| Protein-fat 0.89 occurring with copper refraction | of 38.3 | 16 |
| Protein-fat 0.89 occurring with solids | of 11.42 | 30 |
| Protein-fat 0.89 occurring with fat | of 3.00 | 55 |
| Copper refraction 38.3 occurring with fat | of 3.00 | 6 |

B, 21 PER CENT SKIMMED

| | | |
|---|---|---|
| Protein-fat 0.96 occurring with copper refraction | of 38.3 | 5 |
| Protein-fat 0.96 occurring with solids | of 11.22 | 11 |
| Protein-fat 0.96 occurring with fat | of 2.80 | 20 |
| Copper refraction 38.3 occurring with fat | of 2.80 | 6 |

C, 8 PER CENT SKIMMED

| | | |
|---|---|---|
| Protein-fat 0.91 occurring with copper refraction | of 38.0 | 15 |
| Protein-fat 0.91 occurring with solids | of 12.10 | 19 |
| Protein-fat 0.91 occurring with fat | of 3.30 | 25 |
| Copper refraction 38.0 occurring with fat | of 3.30 | 26 |

D, 14 PER CENT SKIMMED

| | | |
|---|---|---|
| Protein-fat 0.96 occurring with copper refraction | of 38.0 | 6 |
| Protein-fat 0.96 occurring with solids | of 11.90 | 8 |
| Protein-fat 0.96 occurring with fat | of 3.10 | 12 |
| Copper refraction 38.0 occurring with fat | of 3.10 | 14 |

E, 20 PER CENT SKIMMED

| | | |
|---|---|---|
| Protein-fat 0.89 occurring with copper refraction | of 39.5 | 10 |
| Protein-fat 0 89 occurring with solids | of 12.50 | 24 |
| Protein-fat 0.89 occurring with fat | of 3.20 | 34 |
| Copper refraction 39.5 occurring with fat | of 3.20 | 0.6 |

F, 25 PER CENT SKIMMED

| | | |
|---|---|---|
| Protein-fat 0.96 occurring with copper refraction | of 39.5 | 4 |
| Protein-fat 0.96 occurring with solids | of 12.30 | 8 |
| Protein-fat 0.96 occurring with fat | of 3.00 | 22 |
| Copper refraction 39.5 occurring with fat | of 3.00 | 0.07 |

In the cases of examples E and F, the very low frequency of these copper-refraction and fat figures occurring at the same time, taken with the other data, is sufficient evidence to call the samples skimmed with-

out any other data. In the cases of A and B, notwithstanding the high frequency of the occurrence of the observed protein-fat ratio compared with the fat, such samples may be declared skimmed if obtained from one dealer in sufficient quantities to overcome the probabilities of the copper-refraction-fat comparison and the copper-refraction comparison with the protein-fat ratio being natural. In the case of example C, the sale of such milk should be almost a continual performance before skimming could be proved and in the case of example D, at least 15 per cent of the samples obtained from the dealer should be like the example.

## CONCLUSIONS.

A protein-fat ratio of less than 1.0 is no criterion that milk is not adulterated.

The protein-fat ratio is a function of the solids, fat and serum refraction, as well as of the breed, and when less than 1.0, if used in the interpretation of analyses, should be studied in relation to such figures of which it is a function.

Milk representing the mixed milk of many dairies can be declared skimmed when the protein-fat ratio is less than 1.0; provided, however, that other analytical data are obtained to substantiate the conclusion; and provided, further, that a sufficient number of samples has been obtained to exclude the probability of the natural occurrence of such milk.

Owing to the greater prevalence of high protein-fat ratios compared with low protein-fat ratios in milk from the average dairy herds, it is inaccurate to assume that the mixed milk of a number of herds would not greatly exceed in protein-fat ratio that of the average protein-fat ratio of the analyses on record.

In comparing the composition of milk from individual cows with milk from herds, both the maximum and minimum figures obtained from individual cows, as a rule, are not found in herd milk; the protein-fat ratio, however, is an exception for but few of the highest figures so disappear because of greater frequency of protein-fat ratios above the average.

## FIRST DAY.

# MONDAY—MORNING SESSION.

The thirty-sixth annual convention of the Association of Official Agricultural Chemists was called to order by the President, H. C. Lythgoe, of Boston, Mass., on the morning of November 15, 1920, at 10.00 o'clock at the New Willard, Washington, D. C.

## REPORT ON WATER.

By J. W. SALE (Bureau of Chemistry, Washington, D. C.), *Referee.*

Last year a method for the determination of iodine in the presence of chlorine and bromine[1] and a method for the determination of bromine in the presence of chlorine but not iodine[2] were tested, and adopted by the association as tentative methods.

This year a method for the determination of bromine in the presence of both chlorine and iodine[3] has been tested and is as follows:

### BROMINE IN THE PRESENCE OF CHLORINE AND IODINE[3].

#### APPARATUS.

Two, tall form, glass-stoppered Dreschel gas washing cylinders.

An ordinary glass cylindes.

Joined as in Fig. 1.

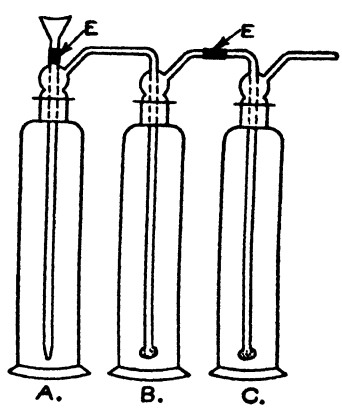



FIG. 1. APPARATUS.
A—Reaction Cylinder. B and C—Absorption Cylinders. E—Rubber Connections.

#### REAGENTS.

(a) *Ferric sulfate crystals.*

(b) *Alkaline sodium sulfite solution.*—Dissolve 4 grams of sodium sulfite and 0.8 gram of sodium carbonate in 100 cc. of water.

(c) *Chromium trioxide crystals.*

(d) *3% solution of hydrogen peroxide.*

(e) *Potassium iodide crystals.*

(f) *N/20 thiosulfate.*

#### REACTIONS.

$Fe_2(SO_4)_3 + 2\,KI = 2\,FeSO_4 + I_2 + K_2SO_4$.

$2\,CrO_3 + 6\,HBr = Cr_2O_3 + 3\,H_2O + 3\,Br_2$.

$2\,H_2CrO_4 + 3\,H_2O_2 = Cr_2O_3 + 3O_2 + 5\,H_2O$.

$Na_2SO_3 + 2\,Br + H_2O = 2\,HBr + Na_2SO_4$.

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[1] *J. Assoc. Official Agr. Chemists*, 1921, **4**: 380.
[2] *Ibid.*, 381.
[3] *J. Ind. Eng. Chem.*, 1920, **12**: 358.

DETERMINATION.

Introduce 10 cc. of the sample into a distillation flask, adjust the volume to about 75 cc. and add 1.5–2.0 grams of ferric sulfate. Distil off the liberated iodine with steam, discarding the distillate. Empty the residue in the distillation flask into a beaker; heat to boiling, add a few drops of methyl orange, and precipitate the iron with ammonia. Avoid an excess of ammonia, as a precipitate of calcium oxide is bulky and difficult to wash. Filter off the iron hydroxide; wash with hot water, and evaporate the filtrate to dryness, or nearly so. During the evaporation do not allow the solution to become acid from the hydrolysis of magnesium chloride.

From this point, proceed as described in the method for bromine in the presence of chlorine but not iodine[1], beginning under "Determination", line 2, "Charge the reaction cylinder A, Fig. 1, by introducing, etc."

Briefly, the method consists of oxidizing iodine with ferric sulfate and distilling off the liberated iodine with steam. After removing iron from the residual liquid, the bromine is oxidized with chromic acid, and the liberated bromine aspirated into an alkaline solution of sodium sulfite. The oxidation is repeated and the bromine aspirated into a solution of potassium iodide, the iodine liberated by the bromine being titrated with a standard solution of sodium thiosulfate.

Synthetic samples of brine were forwarded to eleven analysts who had expressed a desire to cooperate. However, only five submitted their results in time for this report.

The synthetic brine sent to cooperators contained 0.080 gram of bromine per 10 cc. It contained also 0.080 gram of iodine, 1.0 gram of sodium chloride, 0.2 gram of calcium chloride and 0.1 gram of magnesium chloride per 10 cc. of sample.

The data obtained, expressed in grams per 10 cc. of brine, are contained in Table 1.

TABLE 1.

*Bromine obtained by cooperators in synthetic sample of brine.*

| W. E. SHAEFER * | DEARBORN CHEMICAL CO. † | J. G. FAIRCHILD ‡ | C. H. BADGER * | J. W. SALE |
|---|---|---|---|---|
| 0.0740 | 0.0717 | 0.0742 | 0.0756 | 0.0789 |
| 0.0787 | 0.0713 | 0.0748 | 0.0765 | 0.0768 |
| 0.0758 | 0.0717 | ..... | 0.0761 | 0.0782 |
| 0.0781 | 0.0717 | ..... | 0.0765 | ..... |
| 0.0746 | ..... | ..... | ..... | ..... |
| 0.0772 | ..... | ..... | ..... | ..... |
| 0.0785 | ..... | ..... | ..... | ..... |

* Bureau of Chemistry, Washington, D. C.
† Chicago, Ill.
‡ Geological Survey, Washington, D. C.

[1] *J. Assoc. Official Agr. Chemists*, 1921, 4: 381.

| | |
|---|---|
| Average | 0.0755 |
| Theory | 0.0800 |
| Error | 5.6 per cent. |
| Maximum error | 10.9 per cent. |
| Minimum error | 1.4 per cent. |

These results are not so satisfactory as the authors of the method obtained. In fourteen tests the authors recovered 98.2 per cent of the theoretical amount of bromine, whereas our cooperative work indicates that about 95 per cent of the bromine present is recovered.

The method suggests that about 1 hour is sufficient time to aspirate the second time, but it was found that this time should be increased to at least 2 hours. This modification, however, did not result in the recovery of all of the bromine present in the sample.

While it is appreciated that the determination of bromine in mineral waters and brines is beset with difficulties, and that as good results can be obtained by this method as by any in the literature, yet the referee hesitates to recommend it for adoption by the association in view of the results obtained, and especially since the methods for the mineral analysis of water and brine heretofore adopted by the association are capable of giving very accurate results. The low results obtained are probably inherent in the method, and the referee doubts if they can be corrected by modifications. It is recommended, therefore, that instead of adopting this method at the present time, the following statement be inserted in the methods of analysis:

"A volumetric method for the determination of bromine in the presence of chlorine and iodine has been published[1]. Cooperative work indicates that this is probably the best method for bromine which has been published, but the results obtained show that only about 95 per cent of the bromine present is recovered, when 80 mg. of bromine are contained in the portion of the sample used for analysis. The method is satisfactory in the absence of iodine, as shown by the cooperative work on water in 1919."

Another method for bromine[2], which depends upon the oxidation of bromine with chlorine water, subsequent distillation of the bromine formed, and finally titration with standard thiosulfate solution, was tested by W. E. Shaefer at the request of the referee. The results obtained on the sample of synthetic brine used in this cooperative work were inconclusive. The determination of bromine by this method is rapid, and the referee hopes to do some additional work on it.

A method for the estimation of nitrates in water[3] was recently published. The official method[4] of this association involves the removal of chlorine from the sample by means of standard silver sulfate and sub-

[1] *J. Ind. Eng. Chem.*, 1920, 12: 358.
[2] *Z. anal. Chem.*, 1900, 39: 81.
[3] *Analyst.*, 1919, 44: 281.
[4] *Assoc. Official Agr. Chemists, Methods.* 2nd ed., 1920, 23.

sequent treatment of the dried residue with phenoldisulfonic acid reagent. The new method provides for the addition of a diluted phenoldisulfonic acid reagent to the sample before evaporation, which is said to obviate the necessity of the removal of chlorine, thus appreciably reducing manipulation. In the routine work in the referee's laboratory, 69 samples of water were examined, both by the official method and by the new method for nitrate. The two methods gave results which checked in only four cases, the new method giving higher results in 42 cases and lower results in 23 cases, the discrepancy being very large in most instances. A brown coloration was observed frequently on samples run by the new method. This made the colorimetric comparison very unsatisfactory. Under the circumstances, it is believed we are not justified in doing any further work on the new method for nitrate until it has been modified by individual workers.

Except to recommend a continuation of the work on bromine, the referee will not limit the activities of his successor.

## REPORT ON TANNING MATERIALS AND LEATHER.

By F. P. VEITCH (Bureau of Chemistry, Washington, D. C.), *Referee.*

Your referee has no formal report to make. No cooperative work could be undertaken. Work has been done, however, on a problem which is of the greatest importance in the analysis of tanning materials and of leather, and in which the association may also be interested because it is equally important in all analytical work, especially in the determination of moisture or of total solid matter.

It has long been known to analysts that the determination of moisture in finely ground fibrous or fluid materials is difficult and that rarely two analysts, operating at different places or the same analyst operating under different conditions, can get closely agreeing results.

For several years following the experience of the writer in the effect of atmospheric humidity on the physical testing of paper, leather, etc., he has been of the opinion that the relative humidity of the air has a great deal to do with the determination of moisture and of total solids, and experiments thus far conducted, while they can not be regarded as finally conclusive, are indicative that the higher the atmospheric humidity at the time a determination is made, the lower the apparent moisture or the higher the total solids determination will be. If, however, the relative humidity of the air remains constant, closely agreeing results can be obtained repeatedly and, if there are losses, they are progressive and the percentage of moisture or total solids does not fluctuate under these conditions as it does when the material is first dried at low humid-

ity and then at high humidity, and again at low humidity, etc. In other words, the percentage of moisture or of solids in a material fluctuates just as the relative atmospheric humidity existing at the time the moisture determination is made fluctuates and this fluctuation exists to a lesser degree even when the materials are dried at reduced pressure.

It is not necessary to point out, of course, how important this fact, if it is a fact, is in all lines of analytical work. It certainly accounts, in part, at least, for many of the discrepancies which have heretofore been obtained in the work on the determination of moisture and of solids. It is of the highest importance in the analysis of tanning extracts and in the analysis of leather, where a difference of 2 mg. in the weight of the dried material will make an error of 0.3 per cent on a solid extract.

## REPORT ON INSECTICIDES AND FUNGICIDES.

By J. J. T. GRAHAM (Bureau of Chemistry, Washington, D. C.), *Referee.*

The cooperative work included a study of methods for the determination of total arsenic and arsenious oxide in Paris green; for the determination of total arsenic, arsenious oxide, water-soluble arsenic and calcium oxide in calcium arsenate containing calcium arsenite; for the determination of arsenious oxide, water-soluble arsenic and zinc oxide in zinc arsenite; for the determination of total arsenic in London purple; and for the determination of lead oxide, zinc oxide and copper in Bordeaux-lead arsenate-zinc arsenite.

Some of these methods were tested last year, while others are being considered for the first time this year.

Thirty laboratories were invited to cooperate in the work. Of these, six promised to assist in part of the work, and results have been received from ten analysts in three different laboratories.

The following methods were tested:

### PARIS GREEN.

#### TOTAL ARSENIC.

##### REAGENTS.

(a) *Starch indicator.*—Prepare as directed under Paris green[1].

(b) *Standard arsenious oxide solution.*—Prepare as directed under Paris green[1].

(c) *Standard iodine solution.*—Prepare as directed under Paris green[1].

(d) *Standard potassium bromate solution.*—Dissolve 1.688 grams of pure potassium bromate in water and dilute to 1 liter. One cc. of this solution is approximately equal to 0.00300 gram of arsenious oxide. To standardize, transfer 25 cc. aliquots of the standard arsenious oxide solution to 500 cc. Erlenmeyer flasks, add 15 cc. of hydro-

[1] *Assoc. Official Agr. Chemists, Methods.* 2nd ed., 1920, 53.

chloric acid (sp. gr. 1.19), dilute to 100 cc., heat to 90°C. and titrate with the bromate solution, using methyl orange as indicator. The indicator should not be added until near the end of the titration, and the flask should be rotated continuously in order to avoid any local excess of the bromate solution. The end point is shown by a change from red to colorless, and the bromate should be added very cautiously when approaching the end of the titration.

(e) *Standard potassium iodate solution.*—Dissolve 3.244 grams of pure potassium iodate in water and dilute to 1 liter. One cc. of this solution is approximately equal to 0.00300 gram of arsenious oxide. To standardize, transfer 25 cc. aliquots of the standard arsenious oxide solution to 250–500 cc. glass-stoppered bottles, add 55 cc. of hydrochloric acid (sp. gr. 1.19), and dilute to 100 cc. Add 5 cc. of chloroform, and titrate with the iodate solution until the iodine disappears from the chloroform when the mixture is thoroughly shaken.

DETERMINATION.

*Official Distillation Method.*

Proceed as directed under Paris green[1].

*Bromate Method*[2].

Proceed as directed under the official distillation method, using an amount of the sample equal to the arsenious oxide equivalent of 250 cc. of the standard potassium bromate solution, until the distillate is made to volume in a liter graduated flask.

(1) Transfer 200 cc. aliquots of the distillate to 500 cc. Erlenmeyer flasks, heat to 90°C. and titrate with the standard potassium bromate solution, using methyl orange as indicator. The indicator should not be added until near the end of the titration, and the solution should be rotated continuously in order to avoid any local excess of the bromate solution.

(2) Proceed as in (1) with the exception that the titration is made without heating the solution.

The number of cc. of standard bromate solution used, multiplied by 2, represents the per cent of total arsenic in the sample, expressed as arsenious oxide.

*Iodate Method.*

Proceed as directed under the official distillation method, using an amount of the sample equal to the arsenious oxide equivalent of 250 cc. of the standard potassium iodate solution, except that the distillate is made to a volume of 500 cc. Transfer 100 cc. aliquots of the distillate to 250–500 cc. glass-stoppered bottles, add 5 cc. of chloroform and 10 cc. of hydrochloric acid (sp. gr. 1.19), and titrate with the standard iodate solution until the iodine disappears from the chloroform when the mixture is thoroughly shaken[3]. The number of cc. of standard iodate solution used, multiplied by 2, represents the per cent of total arsenic in the sample, expressed as arsenious oxide.

ARSENIOUS OXIDE.

DETERMINATION.

*C. C. Hedges' Method Modified.*

Proceed as directed under Paris green[4].

*Bromate Method.*

Weigh an amount of the sample equal to the arsenious oxide equivalent of 250 cc. of the standard bromate solution, wash into a 250 cc. volumetric flask with 100 cc. of hydrochloric acid (1 to 3), heating to a maximum of 90° C. if necessary to get the sample completely dissolved. Cool, and make to volume.

(1) Transfer a 50 cc. aliquot to a 500 cc. Erlenmeyer flask, add 10 cc. of hydrochloric acid (sp. gr. 1.19), heat to 90°C. and titrate with the standard bromate solution as directed under total arsenic.

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[1] *Assoc. Official Agr. Chemists, Methods.* 2nd ed., 1920, 54.
[2] *Z. anal. Chem.*, 1893, **32**: 415; *J. prakt. Chem.*, 1915, **91**: 133.
[3] *J. Am. Chem. Soc.*, 1903, **25**: 756; *J. Ind. Eng. Chem.*, 1918, **10**: 291.
[4] *Assoc. Official Agr. Chemists, Methods.* 2nd ed., 1920, 55.

(2) Proceed as in (1) with the exception that the titration is made without heating the solution.

The number of cc. of the bromate solution used, multiplied by 2, represents the per cent of arsenious oxide in the sample.

*Iodate Method.*

Weigh an amount of the sample equal to the arsenious oxide equivalent of 250 cc. of the standard iodate solution, wash into a 250 cc. volumetric flask with 100 cc. of hydrochloric acid (1 to 3), heating to a maximum of 90° C. if necessary to get the sample completely dissolved. Cool, and make to volume. Transfer a 50 cc. aliquot to a 250–500 cc. glass-stoppered bottle, add 50 cc. of hydrochloric acid (sp. gr. 1.19), and titrate with the standard iodate solution as directed under total arsenic. The number of cc. of iodate solution used, multiplied by 2, represents the per cent of arsenious oxide in the sample.

TABLE 1.

*Cooperative results on Paris green.*

| ANALYST | TOTAL ARSENIC CALCULATED AS ARSENIOUS OXIDE | | | | ARSENIOUS OXIDE | | | |
|---|---|---|---|---|---|---|---|---|
| | Official Distillation Method | Hot Bromate Method | Cold Bromate Method | Iodate Method | Hedges' Modified Method | Hot Bromate Method | Cold Bromate Method | Iodate Method |
| | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* |
| F. L. Hart, Bureau of Chemistry, Washington, D. C. | 54.60 | 54.43 | 54.43 | 54.46 | .... | 54.47 | 54.43 | 54.56 |
| | 54.69 | 54.47 | 54.43 | 54.46 | .... | 54.49 | 54.43 | 54.56 |
| | .... | .... | .... | 54.51 | .... | .... | .... | .... |
| Average | 54.65 | 54.45 | 54.43 | 54.48 | .... | 54.48 | 54.43 | 54.56 |
| J. J. T. Graham | 54.25 | 54.35 | 54.35 | 54.30 | 54.21 | 54.35 | 54.44 | 54.60 |
| | 54.34 | 54.44 | 54.35 | 54.30 | 54.11 | 54.35 | 54.35 | 54.60 |
| | .... | 54.35 | 54.35 | . . . | 54.25 | 54.35 | 54.54 | 54.50 |
| Average | 54.30 | 54.38 | 54.35 | 54.30 | 54.19 | 54.35 | 54.44 | 54.57 |
| General Average | 54.47 | 54.41 | 54.38 | 54.41 | 54.19 | 54.40 | 54.44 | 54.56 |

COMMENTS BY ANALYST.

*J. J. T. Graham.*—All of the methods checked very closely. The reaction is slow in the cold bromate method and the titrating solution must be added very slowly when the arsenic solution is nearly all oxidized, otherwise the end point may be passed. The hot bromate method is very satisfactory. The iodate method gives accurate results, but the manipulation is not so smooth and easy as in the bromate method.

DISCUSSION.

The bromate and iodate methods for total arsenic both gave excellent results and checked the official distillation method quite closely. These methods also proved satisfactory when applied to the determination of arsenious oxide. A full discussion of these methods will be given under calcium arsenate.

## CALCIUM ARSENATE.

The following methods were tested:

### TOTAL ARSENIC.

#### REAGENTS.

(a) *Starch indicator.*—Prepare as directed under Paris green[1].

(b) *Standard arsenious oxide solution.*—Prepare as directed under Paris green[1]. To convert arsenious oxide to arsenic oxide use the factor 1.16168.

(c) *Standard iodine solution.*—Prepare as directed under Paris green[1].

(d) *Standard potassium bromate solution.*—Dissolve 1.688 grams of pure potassium bromate in water, dilute to 1 liter, and standardize as directed under Paris green, page 33.

(e) *Standard potassium iodate solution.*—Dissolve 3.244 grams of pure potassium iodate in water, dilute to 1 liter, and standardize as directed under Paris green, page 34.

#### DETERMINATION.

*Official Distillation Method.*

Proceed as directed under Paris green[2], using an amount of the sample equal to the arsenic oxide equivalent of 500 cc. of the standard iodine solution and titrating 200 cc. of the distillate. The number of cc. of standard iodine solution used represents directly the total per cent of arsenic in the sample, expressed as arsenic oxide.

*Bromate Method*[3].

Proceed as directed under the official distillation method until the distillate is made to volume in a liter graduated flask, using an amount of the sample equal to the arsenic oxide equivalent of 500 cc. of the standard potassium bromate solution.

(1) Transfer 200 cc. aliquots of the distillate to 500 cc. Erlenmeyer flasks, heat to 90° C. and titrate with the standard potassium bromate solution, using methyl orange as indicator. The indicator should not be added until near the end of the titration, and the solution should be rotated continuously in order to avoid any local excess of the bromate solution.

(2) Proceed as in (1) with the exception that the titration is made without heating the solution.

The number of cc. of the standard bromate solution used, represents directly the total per cent of arsenic in the sample expressed as arsenic oxide.

*Iodate Method.*

Proceed as directed under the official distillation method, using an amount of the sample equal to the arsenic oxide equivalent of 500 cc. of the standard potassium iodate solution, with the exception that the distillate is made to a volume of 500 cc. Transfer 100 cc. aliquots of the distillate to 250–500 cc. glass-stoppered bottles, add 5 cc. of chloroform and 10 cc. of hydrochloric acid (sp. gr. 1.19), and titrate with the standard iodate solution until the iodine disappears from the chloroform when the mixture is thoroughly shaken[4]. The number of cc. of standard iodate solution used, represents directly the total per cent of arsenic in the sample, expressed as arsenic oxide.

### ARSENIOUS OXIDE.

#### DETERMINATION.

*Bromate Method.*

Weigh an amount of the sample equal to the arsenious oxide equivalent of 300 cc. of the standard potassium bromate solution. Transfer to a 500 cc. Erlenmeyer

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[1] *Assoc. Official Agr. Chemists, Methods.* 2nd ed., 1920, 53.
[2] *Ibid.*, 54.
[3] *Z. anal. Chem.*, 1893, **32**: 415; *J. prakt. Chem.*, 1915, **91**: 133.
[4] *J. Am. Chem. Soc.*, 1903, **25**: 756; *J. Ind. Eng. Chem.*, 1918, **10**: 291.

flask, dissolve in 25 cc. of hydrochloric acid (sp. gr. 1.19) and dilute to 100 cc. Heat to 90° C. and titrate with standard bromate solution, using methyl orange as indicator. The number of cc. of bromate solution used, divided by 3, gives the per cent of arsenious oxide in the sample.

*Iodate Method.*

Weigh an amount of the sample equal to the arsenious oxide equivalent of 300 cc. of the standard potassium iodate solution. Transfer to a 300–500 cc. glass-stoppered bottle and dissolve in 30 cc. of hydrochloric acid (sp. gr. 1.19), and 20 cc. of water. Add 5 cc. of chloroform and titrate with standard iodate solution, as directed under Paris green. The number of cc. of standard iodate solution used, divided by 3, gives the per cent of arsenious oxide in the sample.

*Tentative Method for Arsenious Oxide in Lead Arsenate.*

Proceed as directed under lead arsenate[1].

WATER-SOLUBLE ARSENIC.

Proceed as directed under lead arsenate[1].

CALCIUM OXIDE.

Dissolve 2.0 grams of the sample in 80 cc. of acetic acid (1 to 3), transfer to a 200 cc. volumetric flask and make to volume. Filter through a dry filter and transfer a 50 cc. aliquot to a beaker; dilute to 200 cc., heat to boiling and precipitate the calcium with ammonium oxalate. Allow the beaker to stand for 3 hours on the steam bath, filter and wash with hot water. Dissolve the precipitate in dilute sulfuric acid and titrate with permanganate, or ignite and weigh as oxide.

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[1] *Assoc. Official Agr. Chemists, Methods.* 2nd ed., 1920, 59.

TABLE

*Cooperative results on calcium*

| ANALYST | TOTAL ARSENIC CALCULATED AS ARSENIC OXIDE | | | |
|---|---|---|---|---|
| | Official Distillation Method | Hot Bromate Method | Cold Bromate Method | Iodate Method |
| | *per cent* | *per cent* | *per cent* | *per cent* |
| R. H. Robinson, Agricultural Experiment Station, Corvallis, Ore. | 44.98<br>44.98<br>44.98 | 44.95<br>45.02<br>44.99 | 44.99<br>44.97<br>44.97 | 44.98<br>44.99<br>44.99 |
| Average | 44.98 | 44.99 | 44.98 | 44.99 |
| F. L. Hart | 44.48<br>44.58<br>..... | 44.61<br>44.72<br>..... | 44.61<br>44.61<br>..... | 44.68<br>.....<br>..... |
| Average | 44.53 | 44.67 | 44.61 | 44.68 |
| M. H. Goodman, Bureau of Chemistry, Washington, D. C. | 44.90<br>44.69 | 44.97<br>44.97 | 44.85<br>44.85 | 44.77<br>44.92 |
| Average | 44.80 | 44.97 | 44.85 | 44.85 |
| C. F. Sheffield, Agricultural College, Miss. | 45.22<br>45.15<br>..... | 44.84<br>44.90<br>45.02 | .....<br>.....<br>..... | 45.27<br>45.27<br>..... |
| Average | 45.19 | 44.92 | ..... | 45.27 |
| J. J. T. Graham | 44.66<br>44.66<br>44.72 | 44.60<br>44.60<br>44.65 | 44.65<br>44.65<br>44.65 | 44.67<br>44.67<br>..... |
| Average | 44.68 | 44.62 | 44.65 | 44.67 |
| H. D. Young, Bureau of Chemistry, Washington, D. C. | .....<br>..... | .....<br>..... | .....<br>..... | .....<br>..... |
| Average | ..... | ..... | ..... | ..... |
| C. M. Smith, Bureau of Chemistry, Washington, D. C. | .....<br>.....<br>..... | .....<br>.....<br>..... | .....<br>.....<br>..... | .....<br>.....<br>..... |
| Average | ..... | ..... | ..... | ..... |
| General Average | 44.83 | 44.83 | 44.78 | 44.92 |

COMMENTS BY ANALYSTS.

*R. H. Robinson.*—Excellent checks were obtained by all methods for both the total arsenic oxide and the total arsenious oxide of the calcium arsenate containing calcium arsenite. No difficulty was experienced with the iodate method. In the bromate method it was observed that when titrating without heating the solution, the standard bromate solution should be added slowly near the end of the titration in order to per-

2.

*arsenate containing calcium arsenite.*

| ARSENIOUS OXIDE | | | WATER-SOLUBLE ARSENIC (METALLIC) | CALCIUM OXIDE | |
|---|---|---|---|---|---|
| Hot Bromate Method | Tentative Lead Arsenate Method | Iodate Method | | Titrated | Ignited |
| *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* |
| 4.78 | 4.79 | 4.78 | 0.31 | 44.50 | .... |
| 4.80 | 4.79 | 4.78 | 0.31 | 44.45 | .... |
| 4.79 | .... | 4.78 | 0.31 | 44.44 | .... |
| 4.79 | 4.79 | 4.78 | 0.31 | 44.46 | .... |
| 4.82 | .... | 4.86 | 0.42 | 43.84 | 45.08 |
| 4.85 | .... | 4.90 | 0.45 | 43.94 | 45.12 |
| .... | .... | 4.87 | .... | .... | .... |
| 4.84 | .... | 4.88 | 0.44 | 43.89 | 45.10 |
| 4.73 | .... | 4.68 | 0.37 | .... | .... |
| 4.73 | .... | 4.70 | 0.35 | .... | .... |
| 4.73 | .... | 4.69 | 0.36 | | .... |
| 4.83 | .... | 4.73 | .... | .... | .... |
| 4.83 | .... | 4.77 | .... | .... | .... |
| .... | .... | .... | .... | .... | .... |
| 4.83 | .... | 4.75 | .... | .... | .... |
| 4.83 | 4.92 | 4.82 | 0.37 | 43.93 | 43.96 |
| 4.82 | 4.96 | 4.82 | 0.37 | 44.00 | 44.24 |
| 4.82 | .... | 4.82 | 0.39 | 44.00 | 44.96 |
| 4.82 | 4.94 | 4.82 | 0.38 | 43.98 | 44.39 |
| .... | .... | .... | .... | 44.08 | .... |
| .... | .... | .... | .... | 44.08 | .... |
| .... | .... | .... | .... | 44.08 | .... |
| .... | .... | .... | .... | 44.00 | 44.45 |
| .... | .... | .... | .... | 44.02 | 44.30 |
| .... | .... | .... | .... | 43.98 | 44.40 |
| .... | .... | .... | .... | 44.00 | 44.38 |
| 4.81 | 4.87 | 4.79 | 0.37 | 44.10 | 44.56 |

mit reaction and allow the methyl orange to bleach; otherwise, slightly higher results may be obtained.

In the determination of water-soluble arsenic, good triplicate results were obtained at 32° C. if controlled in a similar manner.

*C. M. Smith.*—When the acetic acid solutions of the sample were heated before precipitating the calcium with ammonium oxalate, a slight cloudiness developed,

which may mean that something not an oxalate precipitated, giving higher results by ignition than by titration.

*F. L. Hart.*—I checked the bromate titration for total arsenic against both the official iodine titration and the Gooch-Browning method on about 50 samples, and obtained very good checks. Cold titration works as well as hot, provided the end point is approached more slowly.

In regard to the precipitation of calcium in acetic acid, without previous removal of the arsenic, it seems probable that aluminium comes down as an arsenate along with the calcium oxalate. I obtained appreciable amounts of both aluminium and arsenic in the residues from ignitions of the calcium oxalate. This, of course, would not interfere if the oxalate were titrated with potassium permanganate.

## DISCUSSION.

The bromate and iodate methods for total arsenic gave results that checked very closely the official distillation method. While there is a small variation in the analyses between the different cooperators, the results by individual analysts run uniform for the three methods. Such variation as was obtained can be attributed to differences in standardization of the titrating solutions. Jannasch and Seidel[1] found that the end point in the bromate method was most rapid in solutions whose acidity was between 1 and 3N. The referee has confirmed their statement and finds that the best results are obtained in solutions of approximately 2N acidity.

Nissenson and Siedler[2] in using this method for antimony found that titrations in hot and cold solutions lead to the same results, the hot titrations, however, giving a sharper end point. The work of the referee and a number of the cooperating analysts has shown this to be true also when applied to the determination of arsenic. During titrations in the cold solution the bromate must be added slowly toward the end of the titration in order to allow complete reaction or the results obtained will be slightly high.

A good procedure in the application of the bromate method to routine analysis is to have a standard arsenious oxide solution of exactly the same strength as the bromate solution. In this case the titration is carried on until the red color is destroyed, then a few tenths of a cc. of the arsenious oxide solution and more methyl orange are added and the titration carefully completed. The total titration, minus the amount of added arsenious oxide solution, gives the titration due to the arsenic in the sample.

The results for arsenious oxide by all of the methods are good. When the tentative lead arsenate method is applied to the determination of arsenious oxide in calcium arsenate, considerable attention must be given to making the solution, as superheating occurs, due to the presence of the bulky precipitate of calcium sulfate, which may cause violent bumping.

The method tested for water-soluble arsenic is the one already adopted for water-soluble arsenic in a number of arsenicals, and the results on calcium arsenate are good.

The method for calcium oxide gave good checks when the precipitate was titrated, but the results were high when ignited and weighed. F. L. Hart found aluminium and arsenic in the residues after ignition of the calcium oxalate precipitates, and it is also possible that the precipitate may be contaminated with silica. Hart also determined the calcium oxide in this sample by precipitation in ammoniacal solution, after removal of the arsenic, iron and aluminium. His analysis showed 44.07 per cent by titration and 43.96 per cent by ignition.

To eliminate this contamination of the calcium oxalate precipitate, the method has been modified as follows:

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[1] *J. prakt. Chem.*, 1915, 91: 133.
[2] *Chem. Ztg.*, 1903, 27: 749.

Weigh 2 grams of the sample, transfer to a beaker, add 5 cc. of hydrobromic acid (sp. gr. 1.31) and 15 cc. of hydrochloric acid (sp. gr. 1.19) and evaporate to dryness to remove arsenic; repeat the treatment; then add 20 cc. of hydrochloric acid and again evaporate to dryness. Take up with water and a little hydrochloric acid, filter into a 200 cc. volumetric flask, and make to volume. Transfer a 50 cc. aliquot to a beaker, add 10 cc. of hydrochloric acid and make slightly alkaline with ammonia, using methyl red as indicator. Heat to boiling and filter. Dissolve the precipitate in a little hydrochloric acid, reprecipitate and filter through the same paper. To the combined filtrates and washings add 20 cc. of acetic acid (1 to 3) and adjust the volume to about 200 cc.; heat to boiling, add ammonium oxalate slowly from a buret and allow to stand for 3 hours on a steam bath. Filter, wash with hot water, ignite and weigh as calcium oxide; or dissolve the precipitate in dilute sulfuric acid, heat and titrate with potassium permanganate.

Analysis of this sample by the referee, using the modified method, gave 43.93 per cent of calcium oxide by titration and 43.91 per cent by ignition. These results agree very closely with those obtained volumetrically by the cooperators and show that the method as tested this year gives accurate results when the calcium oxalate is titrated with permanganate. This method is much shorter than the modification outlined above, and the referee recommends that it be adopted as a tentative method, for volumetric work only.

The referee submits the following table of results comparing the hot bromate method with the official distillation method for total arsenic under routine laboratory conditions. After the distillations were completed, one titration was made by the official iodine method and the other by the bromate method.

TABLE 3.

*Comparison of the bromate with the official distillation method for total arsenic.*

(Analyst, F. L. Hart.)

| MATERIAL ANALYZED | BROMATE | OFFICIAL DISTILLATION | VARIATION |
|---|---|---|---|
| | *per cent* | *per cent* | *per cent* |
| Magnesium arsenate | 31.31 | 31.45 | −.14 |
| Magnesium arsenate | 31.86 | 31.96 | −.10 |
| Magnesium arsenate | 31.28 | 31.20 | +.08 |
| Calcium arsenate | 47.61 | 47.71 | −.10 |
| Calcium arsenate | 41.63 | 41.76 | −.13 |
| Calcium arsenate | 46.68 | 46.57 | +.11 |
| Calcium arsenate | 41.95 | 41.89 | +.06 |
| Calcium arsenate | 39.67 | 39.80 | −.13 |
| Calcium arsenate | 16.34 | 16.34 | 0.00 |
| Calcium arsenate | 18.17 | 18.00 | +.17 |
| Calcium arsenate | 38.09 | 38.07 | +.02 |
| Calcium arsenate | 39.70 | 39.60 | +.10 |
| Calcium arsenate | 45.83 | 45.79 | +.04 |
| Calcium arsenate | 42.33 | 42.47 | −.14 |
| Calcium arsenate | 40.25 | 40.24 | +.01 |
| Calcium arsenate | 38.42 | 38.28 | +.14 |
| Calcium arsenate | 34.04 | 34.12 | −.08 |
| Calcium arsenate | 40.25 | 40.24 | +.01 |
| Calcium arsenate | 40.17 | 40.11 | +.06 |
| Calcium arsenate | 41.86 | 41.87 | −.01 |
| Calcium arsenate | 40.81 | 40.70 | +.11 |
| Calcium arsenate | 40.81 | 40.89 | −.08 |
| Lead arsenate | 30.67 | 30.65 | +.02 |
| Calcium-lead arsenate | 16.10 | 16.14 | −.04 |

## BORDEAUX-LEAD ARSENATE-ZINC ARSENITE.

To test the methods for lead oxide, copper and zinc oxide, a sample was prepared by thoroughly mixing 200 grams of Bordeaux mixture, made from commercial copper sulfate and lime, with 100 grams of commercial lead arsenate and 100 grams of commercial zinc arsenite.

The Bordeaux, when analyzed by the official electrolytic method, showed a copper content of 16.18 per cent; the lead arsenate was shown to contain 63.67 per cent of lead oxide by the official sulfate method; while the zinc arsenite contained 57.28 per cent of zinc oxide by the phosphate method.

The mixture submitted for cooperative work should contain, therefore, 8.09 per cent of copper; 15.92 per cent of lead oxide; and 14.32 per cent of zinc oxide.

The directions that were sent out for the determination of these constituents are as follows:

### GENERAL PROCEDURE FOR THE ANALYSIS OF A PRODUCT CONTAINING ARSENIC, ANTIMONY, LEAD, COPPER, ZINC, IRON, CALCIUM, MAGNESIUM, ETC.

(Applicable to such preparations as Bordeaux-lead arsenate; Bordeaux-zinc arsenite; Bordeaux-Paris green; Bordeaux-calcium arsenate; etc.)

#### LEAD OXIDE

Weigh 1 gram of the dry, powdered sample, transfer to a beaker, add 5 cc. of hydrobromic acid (sp. gr. 1.31) and 15 cc. of hydrochloric acid (sp. gr. 1.19) and evaporate to dryness to remove arsenic; repeat the treatment; then add 20 cc. of hydrochloric acid (sp. gr. 1.19) and again evaporate to dryness. Dissolve the residue by heating to boiling in 25 cc. of 2N hydrochloric acid, filter immediately to remove silica, and wash with hot water to a volume of 125 cc. Care must be taken to see that all lead chloride is in solution before filtering. Treat with hydrogen sulfide until precipitation is complete. Filter and wash the precipitate thoroughly with 0.5N hydrochloric acid saturated with hydrogen sulfide. Save the filtrate and washings for the determination of zinc. Transfer the filter paper containing the sulfides of lead and copper to a 400 cc. Pyrex beaker and completely oxidize all organic matter by heating with a few cc. of sulfuric acid, together with a little fuming nitric acid; then completely remove all nitric acid by heating on a hot plate to copious evolution of the white fumes of sulfuric acid, cool, add 2–3 cc. of water and again heat to fuming. Cool and determine the lead as sulfate as directed for lead arsenate[1], beginning with "Cool, add 50 cc. of water and about 100 cc. of 95% alcohol". The alcoholic copper solution should not stand more than 24 hours before filtering, as the solution may creep up the sides of the beaker and deposit crystals of copper sulfate which are very difficult to redissolve in the acid alcohol. From the weight of lead sulfate calculate the amount of lead oxide present, using the factor 0.73600.

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[1] *Assoc. Official Agr. Chemists, Methods.* 2nd ed., 1920, 58.

### COPPER.

Evaporate the filtrate and washings from the lead sulfate precipitate to fuming, add a few cc. of fuming nitric acid to destroy organic matter, and continue the evaporation until about 3 cc. of concentrated sulfuric acid remain. Determine the copper by Low's titration method as directed under Bordeaux mixture[1], or by electrolysis as follows:

Take up the sulfuric acid solution with water, add 1 cc. of concentrated nitric acid, and filter if necessary. Make the volume to about 150 cc. and electrolyze as usual.

### ZINC OXIDE.

#### REAGENT.

*Mercury-thiocyanate solution.*—Dissolve 27 grams of mercuric chloride and 39 grams of potassium thiocyanate in 1 liter of water. In lieu of the potassium thiocyanate, 30 grams of ammonium thiocyanate may be used[2].

#### DETERMINATION.

Carefully concentrate the filtrate and washings from the sulfide precipitation by gentle boiling to about 50 cc. or until all hydrogen sulfide is expelled. Cool, dilute to 100 cc., neutralize with ammonia and add 5 cc. of hydrochloric acid (1 to 1) to each 100 cc. of solution. Add 25 cc. of the mercury-thiocyanate reagent per 100 cc. of solution, and stir vigorously until the zinc is precipitated. Allow to stand for at least 1 hour with occasional stirring, filter through a tared Gooch crucible, wash with water containing 20 cc. of the mercury-thiocyanate reagent per liter, dry at 105° C. and weigh. From this weight calculate the zinc oxide, using the factor 0.16331.

NOTE.—Some iron is generally present and during the zinc determination it should be in the ferrous condition. In making the sulfide precipitation the hydrogen sulfide should be passed into the solution for a sufficient time to reduce the iron, in addition to precipitating the copper and lead. The zinc-mercury-thiocyanate precipitate normally is white and it should not contain occluded ferric thiocyanate sufficient to give it more than a faint pink color.

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[1] *Assoc. Official Agr. Chemists, Methods.* 2nd ed., 1920, 62.
[2] *Trans. Am. Inst. Met.*, 1914, **8**: 146; *J. Am. Chem. Soc.*, 1918, **40**: 1036.

TABLE 4.

*Cooperative results on Bordeaux-lead arsenate-zinc arsenite.*

| ANALYST | ZINC OXIDE | LEAD OXIDE | COPPER |
|---|---|---|---|
| | *per cent* | *per cent* | *per cent* |
| C. B. Stone, Bureau of Chemistry, Washington, D. C. | 13.94 | 16.03 | 7.90 |
| | 14.10 | 16.03 | 7.88 |
| | 13.94 | 15.97 | 7.92 |
| | 13.84 | 16.21 | 7.88 |
| | 14.00 | ..... | ..... |
| Average | 13.96 | 16.06 | 7.90* |
| F. L. Hart | 14.00 | 15.92 | 8.04 |
| | 14.04 | 16.12 | ..... |
| Average | 14.02 | 16.02 | 8.04† |
| J. J. T. Graham | 14.05 | 16.04 | 8.00 |
| | 14.03 | 16.06 | 8.04 |
| | 13.97 | 16.16 | 8.00 |
| | 14.08 | 16.00 | 8.04 |
| | 14.04 | 16.06 | ..... |
| | ..... | 16.10 | ..... |
| Average | 14.03 | 16.07 | 8.02* |
| H. D. Young | 13.94 | 16.05 | 8.02 |
| | 13.93 | 16.18 | 8.00 |
| Average | 13.94 | 16.12 | 8.01.‡ |
| General average | 13.99 | 16.07 | 7.97 |
| Calculated value | 14.32 | 15.92 | 8.09 |

* Determined electrolytically.
† Determined by precipitating the copper as sulfide, igniting and weighing as copper oxide.
‡ Determined by Low's titration method

## DISCUSSION.

The methods for lead oxide and copper are essentially the same as those sent out by the previous referee, while the mercury-thiocyanate method for zinc oxide was tested this year for the first time. The method of preparing the solution has been amended to eliminate any silica that may be present and cause the results for lead oxide to be slightly high. (With this modification care must be taken to see that the lead chloride is all dissolved in the 2N hydrochloric acid before filtering from the silica, or a loss may occur at this point. When large amounts of lead oxide are present it may be necessary to use twice the quantity of 2N acid, and dilute accordingly before precipitating the lead with hydrogen sulfide.) The condition of acidity during the hydrogen sulfide precipitation should be kept within narrow limits, as stated by the referee in 1916[1], in order to insure complete precipitation of the lead without contamination with zinc sulfide. The results obtained by the different analysts on this sample agree

[1] *J. Assoc. Official Agr. Chemists*, 1920, 3: 338.

very well, and show that the methods give excellent results when closely followed. In the zinc determination it is important that the solution be allowed to cool to room temperature before adding the mercury-thiocyanate reagent, and the precipitate should not be washed more than four or five times.

## LONDON PURPLE.

The methods tested are as follows:

### TOTAL ARSENIC.

#### REAGENTS.

(a) *Standard solutions.*—As given under Paris green[1].

(b) *Zinc oxide-sodium carbonate mixture.*—Four parts of zinc oxide and one part of dry sodium carbonate.

(c) *Blood charcoal.*

#### DETERMINATION.

*Official Iodine Method.*

Determine as directed under London purple[2].

*Zinc Oxide-Sodium Carbonate Method.*

Weigh an amount of the sample equal to the arsenious oxide equivalent of 250 cc. of the standard iodine solution. Mix the sample thoroughly with several times its weight of the zinc oxide-sodium carbonate mixture in a shallow porcelain crucible, and cover with a layer of the same mixture. Place the crucible, uncovered, in a muffle; heat, gently at first and finally for about 15 minutes at full heat. The mass will not sinter. Cool, transfer to a distillation flask and proceed according to the official distillation method for total arsenic in Paris green.

*Adsorption Method.*

Proceed as directed under the official iodine method for total arsenic in Paris green, except that 3–4 grams of blood charcoal are added to the flask before beginning the distillation.

*Official Distillation Method.*

Determine as directed under Paris green[3].

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[1] *Assoc. Official Agr. Chemists, Methods.* 2nd ed., 1920, 53.
[2] *Ibid.*, 56.
[3] *Ibid.*, 54.

TABLE 5.

*Cooperative results on London purple.*

(Total arsenic calculated as arsenious oxide.)

| ANALYST | OFFICIAL IODINE METHOD | OFFICIAL DISTILLATION METHOD | ADSORPTION METHOD | ZINC OXIDE-SODIUM CARBONATE METHOD |
|---|---|---|---|---|
| | *per cent* | *per cent* | *per cent* | *per cent* |
| C. B. Stone | 29.64 | 29.81 | 29.69 | 29.77 |
| | 29.64 | 29.81 | 29.80 | 29.77 |
| | ..... | 29.70 | ..... | 29.77 |
| Average | 29.64 | 29.77 | 29.75 | 29.77 |
| | 29.44 | 29.44 | ..... | 29.39 |
| J. K. Dickerson, Bureau of Chemistry, Washington, D. C. | 29.36 | 29.44 | ..... | 29.39 |
| | 29.44 | ..... | ..... | ..... |
| | 29.51 | ..... | ..... | ..... |
| Average | 29.44 | 29.44 | ..... | 29.39 |
| R. F. Russ, Bureau of Chemistry, Washington, D. C. | ..... | 29.66 | 29.69 | 29.84 |
| | ..... | 29.66 | 29.69 | 29.84 |
| | ..... | ..... | ..... | 29.84 |
| Average | ..... | 29.66 | 29.69 | 29.84 |
| General Average | 29.51 | 29.65 | 29.72 | 29.70 |

## DISCUSSION.

The results submitted by the analysts show good agreement by all of the methods.

There has been some objection to the use of the official distillation method for London purple on the ground that dyes are carried over into the distillate, coloring it somewhat and interfering with the titration. The referee has never experienced any difficulty with this method. While the results by the blood charcoal adsorption method agree very closely with those by the other methods, the referee does not feel that this method should be made official. He has found considerable variation in the results for total arsenic on the same London purple when different lots of blood charcoal were used, and it is quite possible that some arsenic may be held in the distillation flask by the charcoal.

## ZINC ARSENITE.

The methods tested are as follows:

### ZINC OXIDE.

#### REAGENT.

Dissolve 27.0 grams of mercuric chloride and 39 grams of potassium thiocyanate in 1 liter of water. In lieu of the potassium thiocyanate, 30 grams of ammonium thiocyanate may be used.

DETERMINATION.

*Mercury-Thiocyanate Method*[1].

Weigh 2.0 grams of the sample and transfer to a beaker. Dissolve in 20 cc. of concentrated hydrochloric acid, wash into a 200 cc. volumetric flask, and dilute to volume. Thoroughly mix the solution and filter through a dry filter. Transfer a 25 cc. aliquot to a beaker and add 5 cc. of concentrated hydro chloricacid. If there is much iron present it should be reduced at this point by adding a little sodium bisulfite and heating on the steam bath until the odor of sulfur dioxide has largely disappeared. Cool, dilute to about 100 cc. and add 35–40 cc. of the mercury-thiocyanate reagent with vigorous stirring. The acid concentration at this point must not exceed 5 per cent. Allow to stand for at least 1 hour, stirring occasionally; filter through a tared Gooch crucible, wash with water containing 20 cc. of the mercury-thiocyanate reagent per liter, dry at 105°C., and weigh. From this weight calculate the per cent of zinc oxide in the sample, using the factor 0.16331.

## ARSENIOUS OXIDE.

REAGENTS.

(a) *Starch indicator.*—Prepare as directed under Paris green[2].

(b) *Standard arsenious oxide solution.*—Prepare as directed under Paris green[2].

(c) *Standard iodine solution.*—Prepare as directed under Paris green[2].

(d) *Standard potassium bromate solution.*—Prepare as directed under Paris green, page 33.

(e) *Standard potassium iodate solution.*—Prepare as directed under Paris green, page 34.

DETERMINATION.

*Bromate Method.*

Transfer a 25 cc. aliquot of the solution, prepared for the determination of zinc, to a 500 cc. Erlenmeyer flask, add 20 cc. of concentrated hydrochloric acid and dilute to 100 cc. Heat to 90° C. and titrate with standard bromate solution, as directed under Paris green.

*Iodate Method.*

Transfer a 25 cc. aliquot of the solution, prepared for the determination of zinc, to a 300–500 cc. glass-stoppered bottle, add 30 cc. of concentrated hydrochloric acid and 5 cc. of chloroform, and titrate with standard iodate solution, as directed under Paris green.

*C. C. Hedges' Method Modified.*

Proceed as directed under Paris green[3].

## WATER-SOLUBLE ARSENIC.

Proceed as directed under lead arsenate[4].

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[1] *Trans. Am. Inst. Met.*, 1914, 8: 146; *J. Am. Chem. Soc.*, 1918, 40: 1036.
[2] *Assoc. Official Agr. Chemists, Methods.* 2nd ed., 1920, 53.
[3] *Ibid.*, 55.
[4] *Ibid.*, 59.

TABLE 6.

*Cooperative results on zinc arsenite.*

| ANALYST | ZINC OXIDE | ARSENIOUS OXIDE | | | WATER-SOLUBLE ARSENIC (METALLIC) |
|---|---|---|---|---|---|
| | | BROMATE METHOD | IODATE METHOD | HEDGES' METHOD MODIFIED | |
| | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* |
| R. H. Robinson | 56.49 | 40.58 | 40.57 | .... | Trace, less than 0.01 |
| | 56.39 | 40.58 | 40.57 | .... | |
| | 56.46 | .... | .... | .... | |
| | 56.47 | .... | .... | .... | |
| | 56.38 | .... | .... | .... | |
| | 56.41 | .... | .... | .... | |
| Average | 56.43 | 40.58 | 40.57 | .... | Trace |
| F. L. Hart | .... | 40.08 | 40.05 | 39.95 | 0.08 |
| | .... | 40.14 | 40.00 | .... | 0.08 |
| | .... | .... | .... | .... | 0.08 |
| Average | .... | 40.11 | 40.03 | 39.95 | 0.08 |
| C. F. Sheffield | 56.36 | .... | .... | .... | ..... |
| | 56.44 | .... | .... | .... | ..... |
| Average | 56.40 | .... | .... | .... | ..... |
| H. D. Young | 56.67 | 39.88 | 39.93 | .... | ..... |
| | 56.77 | 39.90 | 39.93 | .... | ..... |
| Average | 56.72 | 39.89 | 39.93 | .... | ..... |
| J. J. T. Graham | 56.92 | 40.07 | 40.20 | 40.00 | 0.02 |
| | 56.97 | 40.01 | 40.08 | 40.00 | 0.02 |
| | 56.99 | 40.07 | 40.08 | 40.06 | ..... |
| | 57.02 | .... | .... | .... | ..... |
| Average | 56.98 | 40.05 | 40.12 | 40.02 | 0.02 |
| General Average | 56.62 | 40.15 | 40.16 | 40.00 | 0.06 |

COMMENTS BY ANALYSTS.

*R. H. Robinson.*—No difficulty was experienced in obtaining check results by the different methods for arsenious oxide in zinc arsenite.

In the determination of zinc in zinc arsenite, potassium thiocyanate was used in the mercury-thiocyanate reagent. Excellent duplicate results were obtained when precipitation of the zinc was made at the same temperature. The results were obtained at 26–28° C., the temperature of the laboratory at the time the determinations were made. It was found, however, that when all solutions were cooled to 15–18° C. and

maintained at that temperature until filtered, the results were about 0.2 per cent higher. Contrary to Jamieson's statement that "drying at 105° C. for 1 hour was sufficient", it was necessary to dry in an electric oven at 105° C. for about 10 hours to obtain constant weight.

## DISCUSSION.

The results for arsenious oxide, with one exception, agree fairly well. While one of the analysts reports slightly higher values than the other cooperators, his results agree very closely by the different methods.

The method for water-soluble arsenic gives good results and is already official for a number of arsenicals.

In the mercury-thiocyanate method for zinc oxide there is some variation in the results by the different analysts, but the different determinations by the individual analysts agree quite closely. One analyst thinks that differences in temperature cause a variation in the results. The referee has found that sets of determinations made at different times, while checking quite well in each set, often vary slightly between sets. He has made a partial study of the cause of this variation, but is not in a position to draw any definite conclusions at present. Drying the precipitate for 1 hour at 105° C. produced constant weight for him in all cases. Analysis of this sample by the phosphate method gave a zinc oxide content of 57.28 per cent, which is slightly higher than the results submitted by any of the analysts.

## RECOMMENDATIONS.

After consultation with Committee A, the referee decided to make no recommendation to the association for the adoption of the iodate method for the determination of total arsenic and arsenious oxide. Although the results by this method are excellent, the bromate method is equally accurate, more easily manipulated, and the materials required are cheaper.

It is recommended—

(1) That the hot bromate method, page 34, be adopted as an official method for the titration of the acid distillate in the official distillation method for the determination of total arsenic.

(2) That the hot bromate method, page 34, for the determination of arsenious oxide in Paris green be adopted as an official method.

(3) That the bromate method for the determination of arsenious oxide in calcium arsenate, page 36, be adopted as an official method.

(4) That the tentative method for the determination of arsenious oxide in lead arsenate[1], be adopted as a tentative method for the determination of arsenious oxide in calcium arsenate.

(5) That the official method for the determination of water-soluble arsenic in lead arsenate, page 37, be adopted as official for the determination of water-soluble arsenic in calcium arsenate.

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[1] *Assoc. Official Agr. Chemists, Methods.* 2nd ed., 1920, 59.

(6) That the modified method for calcium oxide, page 41, be adopted as a tentative method.

(7) That the method for calcium oxide, page 37, be amended by eliminating the words, "or ignite and weigh as oxide", and when so amended that it be adopted as a tentative method.

(8) That in the "General procedure for the analysis of a product containing arsenic, antimony, lead, copper, zinc, iron, calcium, magnesium, etc.", page 42, the methods for lead oxide and copper be adopted as official methods and the method for zinc oxide be adopted as a tentative method.

(9) That the official distillation method[1] be adopted as an official method for the determination of total arsenic in London purple.

(10) That the zinc oxide-sodium carbonate method[2] be adopted as an official method for the determination of total arsenic in London purple.

(11) That the mercury-thiocyanate method for zinc oxide in zinc arsenite, page 47, be adopted as a tentative method.

(12) That the bromate method for the determination of arsenious oxide in zinc arsenite be adopted as an official method.

(13) That the official method for the determination of water-soluble arsenic in lead arsenate, page 47, be adopted as official for the determination of water-soluble arsenic in zinc arsenite.

(14) That the official distillation method[1] be adopted as an official method for the determination of total arsenic in magnesium arsenate.

(15) That a study be made of methods for the determination of arsenious oxide, water-soluble arsenic and magnesium in magnesium arsenate.

## PICKERING BORDEAUX SPRAYS.

By F. C. Cook (Bureau of Chemistry, Washington, D. C.).

The copper in Bordeaux spray has generally been considered to be in the form of copper hydrate. Pickering, an English chemist, studied the composition of Bordeaux and of other sprays, known as Pickering sprays, made by mixing dilute solutions of copper sulfate with lime water. He found the copper in Bordeaux and in the Pickering sprays to be present as basic sulfates of copper, the amount of lime used determining the composition of the basic sulfate. Pickering made laboratory tests, passing carbon dioxide through suspensions of the different sprays, and determined the amount of copper made soluble. He found eight to fifteen times as much copper sulfate re-formed from the different Pickering sprays as from Bordeaux spray. The conclusion was drawn that the

[1] *Assoc. Official Agr. Chemists, Methods.* 2nd ed., 1920, 54.
[2] *J. Assoc. Official Agr. Chemists*, 1921, 4: 397.

Pickering sprays prepared with lime water, in place of milk of lime, were many times more efficacious per unit of copper than the standard Bordeaux sprays.

In 1915, when copper sulfate increased in price to twenty-five and thirty cents a pound in certain parts of this country, C. L. Alsberg and J. K. Haywood of the Bureau of Chemistry arranged with the Bureau of Plant Industry for cooperative tests to determine if the Pickering sprays, which required less copper sulfate than Bordeaux, would give as good control of fungous diseases as obtained with standard Bordeaux. The tests, which extended over three seasons, were made on apples, grapes, cranberries and potatoes.

The cooperation of the Maine Agricultural Experiment Station was secured for the potato tests. The Pickering sprays, because of their caustic properties, can not be recommended for apples in Virginia or for grapes in Virginia and New Jersey. Whether similar results will follow elsewhere can only be determined by tests in different localities. The results on cranberries in New Jersey and on potatoes in Maine were satisfactory. The late blight, *phytophthora infestans*, of potatoes was controlled and the yields increased as with regular Bordeaux. The amount of copper sulfate in the Pickering spray successfully used on potatoes was 46 per cent less than in the 5-5-50 Bordeaux used. The Pickering sprays are easily applied and involve less wear and tear on the apparatus than Bordeaux sprays.

The past two seasons data have been secured which indicate strongly that copper sprays, such as Bordeaux and Pickering, not only increase the yield of tubers but increase the solid matter of the tubers, *i. e.*, increase the food value. The results of the experiments have been published by the Department of Agriculture[1]. It is hoped that the Pickering sprays may be tried on different crops in various parts of the country in order to determine their value.

A barium water spray was also used on apples and potatoes with satisfactory results. Such a spray effected a considerable saving of copper and gave an increased yield of potatoes. It has the advantage of being prepared with barium hydrate, which possesses some slight fungicidal properties and which dissolves more readily than lime in water and leaves no insoluble residue.

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[1] U. S. Dept. Agr. Bull. 866: (1920).

# REPORT ON SOILS.

By W. H. MacIntire (Agricultural Experiment Station, Knoxville, Tenn.), *Referee.*

As directed by the association, the referee has undertaken a study of the sulfur problem. It so happened that this subject has been studied in the laboratory of the referee during the past six years from the viewpoint of native soil sulfur and precipitated sulfur, through the medium of forty-six lysimeters. On the other hand, the problem of added sulfur, in three forms, iron sulfate, iron pyrites and flowers of sulfur, has been under investigation during the past three years through the installation of twenty-two lysimeters, installed primarily for sulfur-addition research.

Since this association directed that the problem be recognized and studied by the referee, the work has been extensively supplemented under laboratory-control conditions where the pure chemistry of sulfur transformations characteristic of soils is being investigated. One assistant has been giving his full time to these laboratory studies and a number of intensely interesting and indicative findings have been recorded. It is hoped that these studies will be completed in time to present the summarization of results at the 1921 meeting of the association.

The referee has devoted some months of study upon an attempt to perfect an analytical method which would recover for determination all of the sulfur content of soils. Since the work, though well advanced, lacks much of completion, it does not seem necessary to burden the association with its details at this time. Briefly summarized, however, it may be said that a number of different procedures were tried upon three soils in an effort to secure a full recovery of added sulfates of sodium and magnesium. The original soil was run parallel with portions impregnated with definite additions, as aliquots of one or more standard solutions. The methods of procedure were as follows:

(1) The magnesium nitrate incineration procedure.

(2) The sodium peroxide incineration and fusion.

(3) Boiling with concentrated nitric acid; filtration through Büchner; addition of bases; evaporation and heating to convert iron salts to oxides, followed by boiling with acetic acid and filtration, to effect solution of sulfates, and removal of iron oxides.

(4) Boiling with aqua regia; filtration through Büchner; addition of bases; evaporation and heating to convert iron salts to oxides, followed by boiling with acetic acid and filtration, to effect solution of sulfates, and removal of iron oxides.

(5) Boiling with nitro-hydrofluoric acid; filtration through Büchner; addition of bases and elimination of hydrofluoric acid by repeated evap-

orations with hydrochloric acid; heating to convert iron salts to oxides, followed by boiling with acetic acid, and filtration to bring sulfates into solution and remove iron oxides.

(6) The J. Lawrence Smith method and bromine additions.

(7) Incineration with calcium and magnesium carbonates and nitrates with bromine addition to solution.

The incinerated residues were extracted separately with water, acetic acid and hydrochloric acid. None of these procedures effected a full recovery of even the added sulfur, much less the total of native and added amounts. Native barium of the soil was found to be one inhibitory factor in the partial recovery of sulfur. The rehydration of the silica by acid additions seemed to be a further, if not major, deterrent to sulfate recovery. The insolubility of ignited sulfates appeared also to contribute to the incomplete leaching of sulfates from the insoluble residues.

The one procedure, as carried out with one or more variations in detail of technique, which produced a total, equivalent to the native and added sulfur, as sulfates, was as follows:

Mix 25-gram charges of soil with ammonium nitrate, ammonium chloride and calcium carbonate by thorough dry pestling. Transfer to two 15 cc. platinum or Vitreosil crucibles and heat in an electric furnace for 1 hour at full heat. After cooling, remove and pulverize, if necessary. Hydrate the calcium oxide and then add water to a volume of about 200 cc. Heat nearly to boiling on an electric plate, or sulfur-free flame, and throw onto a Büchner filter. Wash to a volume of 1 liter. Then add an excess of acetic acid to the residue (volume of 200 cc.); heat and repeat the Büchner filtration; and washing to 1 liter. Evaporate and combine the filtrates. Precipitate barium sulfate from a volume of 400 cc.

NOTE: It has not yet been determined whether it is essential to remove most or all of the calcium and magnesium. This has been done by the addition of ammonium carbonate to the separate water and acetic acid filtrates; the elimination of ammonium chloride by metathesis with nitric acid, followed by two evaporations with hydrochloric acid.

An effort will be made to perfect this tentative procedure.

Since it was impossible to secure an associate of experience who could devote sufficient time to a further study of the lime absorption coefficient, this work was not continued during the past year.

## RECOMMENDATIONS.

It is recommended—

(1) That work be continued during the ensuing year in an effort to perfect the suggested method or some other procedure which will insure the complete recovery of total soil sulfur.

(2) That a committee of three be appointed by the president of this association, to confer with a similar committee, already appointed, from the American Society for Testing Materials, relative to methods of analy-

sis and proposed uniform legislation for the control of the sale of agricultural lime.

No associate referee on sulfur in soils was appointed and no special report on this subject was presented.

## REPORT ON TESTING CHEMICAL REAGENTS.

By W. D. COLLINS (U. S. Geological Survey, Washington, D. C.), *Referee.*

The purity and strength required for reagents to be used in the analytical methods of this association are not markedly different from the requirements for reagents for any other careful analytical work. Therefore there would seem to be no need for extensive study with collaborative work on this subject by the association. The reports of past referees are in harmony with this conclusion.

The worst feature of the situation in regard to reagents is the lack of reliability. This does not seem to be caused so much by lack of methods of testing or lack of knowledge of manufacturing processes, as by failure to keep the purity and reliability up to the standard permitted by the knowledge which is at hand.

Manufacturers and dealers do not have enough specific complaints of low quality to justify them in believing that their products are on the whole much below the standard which may reasonably be expected for a reagent manufactured and sold in a commercial way. They are inclined to believe that on the whole they are doing pretty well. The Committee on Guaranteed Reagents and Standard Apparatus of the American Chemical Society is endeavoring to secure data to show whether manufacturers are justified in this belief and to help members of the society to secure satisfactory reagents. If enough information in regard to specific instances of deliveries of poor reagents can be obtained, it will be possible to direct purchasers to the best sources for individual reagents and to assist manufacturers to bring their standards up to the level of the general demand. In order to help in this work the following recommendations are made:

### RECOMMENDATIONS.

(1) That the association declare itself in favor of cooperating with the Committee on Guaranteed Reagents and Standard Apparatus of the American Chemical Society in the collection of data in regard to the quality of reagents on the market.

(2) That the secretary, or referee on testing chemical reagents, be instructed to transmit a statement of this action to the proper official of each institution represented in the membership of the association

and request that the purchasing agent or some other official of the institution send to the Committee on Guaranteed Reagents and Standard Apparatus of the American Chemical Society a carbon copy of each letter written to a manufacturer or dealer calling attention to a specific instance of delivery of an unsatisfactory reagent.

No report on foods and feeding stuffs was made by the referee.

## REPORT ON CRUDE FIBER.

By G. L. BIDWELL (Bureau of Chemistry, Washington, D. C.), *Referee.*

The referee has had very satisfactory cooperation, and wishes both to thank the cooperators and to ask their cooperation in the work next year. In February samples were sent to about fifty collaborators with the request that the crude fiber be determined by the methods in use at their laboratories; also, that the method used be reported. The methods differed so much that work was started in the referee's laboratory to see what effect these various differences might have. The results of that work are given in a separate paper, page 58.

The results on the first sample are shown in Table 1.

TABLE 1.

*Cooperative results on crude fiber, Sample No. 1.*

| LABORATORY NUMBER | CRUDE FIBER | LABORATORY NUMBER | CRUDE FIBER | LABORATORY NUMBER | CRUDE FIBER |
|---|---|---|---|---|---|
| | *per cent* | | *per cent* | | *per cent* |
| $W_2$ | 8.71 | 1 | 8.77 | 22 | 9.08 |
| $W_3$ | 8.75 | 2 | 8.68 | 23 | 8.74 |
| A | 8.35 | 3 | 8.25 | 24 | 8.25 |
| B | 8.68 | 4 | 7.50 | 25 | 8.19 |
| C | 9.06 | 5 | 7.54 | 26 | 8.81 |
| D | 8.79 | 6 | 8.84 | 27 | 8.66 |
| E | 9.14 | 7 | 8.98 | 28 | 8.43 |
| F | 8.85 | 8 | 8.74 | 29 | 8.52 |
| G | 8.10 | 9 | 8.66 | 30 | 8.33 |
| H | 9.05 | 10 | 8.55 | 31 | 8.62 |
| I | 8.57 | 11 | 8.02 | 32 | 8.77 |
| J | 8.59 | 12 | 8.52 | 33 | 9.48 |
| K | 8.31 | 13 | 8.33 | | |
| L | 7.58 | 14 | 8.51 | Average of all samples | 8.56 |
| M | 8.45 | 15 | 8.36 | | |
| N | 8.79 | 16 | 8.63 | | |
| O | 8.75 | 17 | 8.61 | | |
| P | 8.78 | 18 | 8.47 | | |
| Q | 8.04 | 19 | 9.00 | | |
| R | 8.44 | 20 | 8.48 | | |
| S | 8.44 | 21 | 8.84 | | |

Later, a second sample was sent to a portion of the first list with the same directions. This was a cottonseed meal. The results follow:

TABLE 2.

*Cooperative results on crude fiber, Sample No. 2.*

| LABORATORY NUMBER | CRUDE FIBER | LABORATORY NUMBER | CRUDE FIBER |
|---|---|---|---|
| | *per cent* | | *per cent* |
| W | 12.85 | L | 12.69 |
| $W_2$ | 12.80 | M | 13.40 |
| $W_3$ | 12.89 | N | 12.62 |
| $W_4$ | 12.79 | O | 12.06 |
| A | 13.20 | P | 11.46 |
| B | 12.54 | Q | 11.84 |
| C | 12.62 | R | 11.89 |
| D | 12.83 | S | 12.80 |
| E | 12.09 | | |
| F | 12.12 | Average of all samples | 12.61 |
| G | 12.15 | | |
| H | 11.82 | | |
| I | 13.49 | | |
| J | 13.73 | | |
| K | 13.25 | | |

Still later, a third sample was sent to the full list of collaborators with a method that has been found to give good results. The results obtained are given in Table 3.

TABLE 3.

*Cooperative results on crude fiber, Sample No. 3.*

| LABORATORY NUMBER | CRUDE FIBER | LABORATORY NUMBER | CRUDE FIBER |
|---|---|---|---|
| | *per cent* | | *per cent* |
| $W_1$ | 13.88 | 1 | 13.35 |
| $W_2$ | 13.89 | 2 | 14.09 |
| $W_3$ | 13.90 | 3 | 13.25 |
| $W_4$ | 13.65 | 4 | 13.14 |
| A | 13.44 | 5 | 13.75 |
| B | 13.44 | 6 | 13.86 |
| C | 13.60 | 7 | 15.80 |
| D | 13.65 | 8 | 14.60 |
| E | 14.22 | 9 | 13.51 |
| F | 13.00 | 10 | 14.07 |
| G | 13.21 | 11 | 14.48 |
| H | 15.50 | 12 | 13.05 |
| I | 13.91 | 13 | 13.81 |
| J | 13.01 | 14 | 13.69 |
| K | 12.90 | 15 | 12.75 |
| L | 13.17 | 16 | 12.66 |
| M | 13.02 | 17 | 13.43 |
| N | 13.32 | | |
| O | 13.44 | Average of all samples | 13.65 |

These results vary about as much as those on Sample No. 1, but this can be explained on the basis that it was a more or less new procedure for the analysts. In the referee's opinion, these results do not justify the recommendation that this method be adopted by the association but are good enough to justify a continuation of this work.

The referee has been asked to recommend to the association several additions to the official method. This has not been done for the following reasons: All of the feed methods can be classed as definitive methods, that is to say, the definition of the name of the result is the method used in obtaining that result. For instance, the percentage of crude protein is not the exact percentage of protein by weight in a sample but is the result obtained by multiplying the percentage of nitrogen obtained by an approved method by the factor 6.25. There is no other feed method that is so definitive, however, as the crude fiber method. Almost any change that can be made and still keep within the method as it is at present will make some change in the results. The foregoing tables show how much the values may vary among laboratories that turn out good work. In the opinion of many, this condition will not be improved until the method is so changed that all analysts will run this determination in exactly the same way. To accomplish this, the method must describe and specify each step in detail and must have just as few optional details as possible. In other words, all must use the same kind of condensers, filtering media, flasks, crucibles and the same degree of heat.

When this is done, concordant results will be obtained as is shown by the results marked $W_1$, $W_2$, $W_3$, and $W_4$ in Tables 1, 2, and 3 which are the results obtained by analysts in the Bureau of Chemistry at Washington working wholly independently but using precisely the same method.

The referee plans to try to ascertain the reasons for the differences in Table 3 and, by changing the method, see if better results can not be obtained. Then the referee hopes to be able to recommend a precise method for crude fiber. It is felt that such a method, if followed, will give results that will be more uniform than can be obtained from the present one.

## RECOMMENDATION.

It is recommended that the crude fiber method be further studied.

# A STUDY OF THE DETAILS OF THE CRUDE FIBER METHOD.

By G. L. BIDWELL and L. E. BOPST (Bureau of Chemistry, Washington, D. C.).

The crude fiber question has been under discussion by feed chemists for several years, yet, after all the collaborative work that has been done and all the discussion that has taken place, there still exists much difference of opinion in regard to this matter. Considerable difficulty has been experienced by many analysts in securing concordant and satisfactory results. In view of this, it would seem that the official method[1], adopted years ago, is unsatisfactory. This condition may possibly be due to the fact that the official method is not given in sufficient detail and many chemists introduce variations which have a material effect upon the results of the determination, yet they feel they are still within the bounds of the official method. While some of the trouble may be due to difficulties in manipulation, it would seem that if a method were given in precise terms experienced men would have no trouble in securing concordant results.

This work has brought out the fact that all chemists should be brought to realize that this method is purely a definitive one; that is, crude fiber is defined by the method just as volatile combustible matter in coal is defined by the method, and is *not* the determination of a definite substance like calcium in limestone. It has been found that some chemists seem to think an attempt is being made to determine a definite substance, cellulose. This is not the case, however, for, when cellulose in its various forms is determined, results are obtained which *differ* from those of crude fiber. It follows, then, that the determination must be made by the different chemists in exactly the same way in every detail in order to obtain concordant results, for very slight changes make appreciable differences.

The foremost object of this paper is to demonstrate the effect of small changes that probably come within the bounds of the official method, and to determine what details must be included in a proposed method to make it absolutely satisfactory. A method has been developed, after much work, that will give concordant results if care is taken to follow it exactly. All results tabulated in this paper were obtained by using the following method as a basis, with such variations as may be used specifically stated.

### REAGENTS.

*Dilute sulfuric acid solution.*—Contains 1.25 grams of sulfuric acid per 100 cc.

*Dilute sodium hydroxide solution.*—Contains 1.25 grams of sodium hydroxide per 100 cc., free, or nearly free, from sodium carbonate.

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[1] *Assoc. Official Agr. Chemists, Methods.* 2nd ed., 1921 97

The strength of these solutions should be accurately checked by titration.

*Asbestos.*—Previously treated with acid and alkali and ignited.

APPARATUS.

*Liebig condenser (about 15 inch).*

*Container.*—Any container that will give 1½ inches depth of boiling solution and allow the use of a Liebig condenser.

*Funnel.*—Any ribbed funnel.

*Linen.*—Linen should be of such character that while filtration is rapid practically no solid matter passes through.

DETERMINATION.

Extract 1–2 grams of the dry material with ordinary ether, or the residue from the ether extract determination may be used, and transfer the residue, together with ½-1 gram of asbestos, to the assay flask. Add 200 cc. of boiling 1.25% sulfuric acid and digest for 30 minutes. Filter through linen, wash thoroughly and digest 30 minutes with 1.25% boiling sodium hydroxide. Filter through a prepared Gooch crucible, wash thoroughly, dry, weigh, incinerate and weigh again. The difference in weight is taken as crude fiber.

In undertaking this work the first problem confronted was to obtain a sample that would give the same results day after day; in other words, one that was efficiently ground and thoroughly mixed. After experimenting for some time, a mixture of 25 per cent alfalfa and 75 per cent cornmeal, ground to pass a 40-mesh sieve, was found to give very close checks over a period of several weeks. The results of the series of determinations are shown in the following table:

TABLE 1.

*Individual determinations of crude fiber on test sample.*

| NOVEMBER 3 | NOVEMBER 5 | NOVEMBER 13 | DECEMBER 3 | JANUARY 15 | FEBRUARY 10 |
|---|---|---|---|---|---|
| *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* |
| 9.48 | 9.55 | 9.60 | 9.59 | 9.38 | 9.52 |
| 9.46 | 9.40 | 9.63 | 9.50 | 9.39 | 9.46 |
| 9.44 | .... | . | 9.45 | .... | 9.54 |
| 9.36 | 9.60 | 9.64 | 9.42 | 9.46 | .... |

The average of all the results obtained was 9.50 per cent of crude fiber.

Having prepared a satisfactory sample, the next step was the determination of the efficiency of different condensers. It was found that various types of condensers were being used by the different collaborators and the following list includes most of them:

1. Watch glasses.
2. Round-bottomed flasks containing cold water.
3. Round-bottomed flasks with a stream of cold water passing through.
4. Funnels.
5. Kjeldahl condensing bulbs.
6. Air-cooled glass tubes.

### WATCH GLASSES.

Four determinations on the above-mentioned sample were made according to the method with the exception that a watch glass[1] was used to cover the mouth of the container in place of a Liebig condenser. The following results were obtained: 9.26, 9.11, 9.10 and 9.25 per cent of crude fiber; average, 9.18 per cent. These figures indicate that this modification of the method has caused low results and is undoubtedly due to evaporation, for the stronger the solution the greater the action upon the material.

### ROUND-BOTTOMED FLASKS CONTAINING COLD WATER.

In place of Liebig condensers, bottles filled with cold water were placed upon the container and four determinations made with results as follows: 9.30, 9.27, 9.23 and 9.10 per cent of crude fiber; average, 9.23 per cent. These condensers proved partially satisfactory for the first 10 minutes; then the water in the bottles became warm and there was a concentration of solution, due to the escape of water vapor around the junction of the flask and bottle, causing the low results.

### ROUND-BOTTOMED FLASKS WITH A STREAM OF TAP WATER PASSING THROUGH.

With this variation in the method, the following results were obtained: 9.27, 9.32, 9.30 and 9.28 per cent of crude fiber; average, 9.29 per cent. This possibly is more efficient than other types except, of course, the Liebig. Loss by evaporation and inability to control frothing, which is encountered in some samples, are objections to this modification. Although this type of condenser is used to quite an extent the results show 0.2 per cent less fiber than when Liebig condensers are used.

### FUNNELS.

In testing the efficiency of funnels as condensers, two sets of determinations were made. In one set no attempt was made to keep the volume to 200 cc. and the following results were obtained on four determinations: 9.10, 9.02, 9.11 and 9.00 per cent of crude fiber; average, 9.06 per cent. In the other set, water was occasionally added, in order to maintain the original volume, with the following results: 9.40, 9.32, 9.28 and 9.42 per cent of crude fiber; average, 9.36 per cent.

These two sets of results show conclusively that funnels are unsatisfactory as condensers and that they are the most inefficient of all types investigated. The addition of water helps to a certain extent but results obtained with the use of funnels for condensers will never be so satisfactory as those obtained by the use of Liebig.

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[1] *J. Ind. Eng. Chem.*, 1910, 2: 281.

### KJELDAHL CONDENSING BULBS.

The Kjeldahl condensing bulbs were next tried with the following results: 9.38, 9.40, 9.36 and 9.31 per cent of crude fiber; average, 9.36 per cent.

These results are not so good as obtained with the Liebig condenser.

### AIR-COOLED GLASS TUBES.

Glass tubes about 20 inches long and ¼ inch in diameter were used for condensers with the following results: 9.28, 9.33, 9.36 and 9.26 per cent of crude fiber; average, 9.31 per cent. This type of condenser is, of course, very inexpensive and easy to manipulate. However, the results are lower than those obtained with the Liebig condenser, due to loss by evaporation which causes concentration of solution resulting in additional action on the charge.

From these figures it can be readily seen that condensers play a very important part in this determination. Concentration of solution due to evaporation noticeably lowers the results. To obtain concordant results one type of condenser must be used by all. The Liebig water-jacketed condenser[1] gives the most satisfactory and uniform results of any type used and since it is standard equipment there should be no objection to its use.

The next step was to determine just what effect a delay in the acid and alkali filtration would have upon the results. Delays of 7, 14 and 28 minutes were made on both acid and alkali filtrations.

TABLE 2.

*Crude fiber obtained by a delay in acid filtration.*

| DELAY OF 7 MINUTES | DELAY OF 14 MINUTES | DELAY OF 28 MINUTES |
|---|---|---|
| *per cent* | *per cent* | *per cent* |
| 9.38 | 9.35 | 9.32 |
| 9.32 | 9.38 | 9.26 |
| 9.21 | 9.30 | 9.24 |
| 9.18 | 9.35 | 9.19 |
| .... | 9.42 | .... |
| .... | 9.36 | .... |
| Average. .9.27 | 9.36 | 9.25 |

Where the acid filtration was delayed 7 minutes a lowering of the results is noted. This is due to the additional time that the acid is allowed to act upon the charge. The abnormal results noted in the 14-minute

[1] *J. Ind. Eng. Chem.*, 1910, 2: 281.

periods may be due to a combination of two actions: the further disintegrating action of the acid, due to additional time, which would lower results; and the precipitation of material once dissolved, due to lowering of temperature of solution while standing, which would raise results. The results from the 28-minute period show an additional drop in fiber corresponding somewhat to the 7-minute period. The point emphasized by these results is that unless the solution is filtered immediately after removal from the flame, additional action takes place which noticeably affects the results and no sample requiring more than 5 minutes for acid filtration should be reported.

### DELAY IN ALKALI FILTRATION.

Four determinations were carried through the regular acid and alkali digestion but immediately after the expiration of the 30 minutes' alkali boiling they were allowed to stand for periods of 7, 14 and 28 minutes with the following results:

TABLE 3.

*Crude fiber obtained by a delay in alkali filtration.*

| DELAY OF 7 MINUTES | DELAY OF 14 MINUTES | DELAY OF 28 MINUTES |
|---|---|---|
| *per cent* | *per cent* | *per cent* |
| 9.52 | 9.48 | 9.48 |
| 9.48 | 9.45 | 9.43 |
| 9.44 | 9.51 | 9.51 |
| 9.50 | 9.49 | 9.35 |
| Average..9.49 | 9.48 | 9.44 |

No great difference is noticed in the results by delaying the alkali filtration on this sample[1]. In order to determine whether this variation in the method would give similar results upon a feed having different properties, the same experiment was tried with cottonseed meal. Determinations were made upon a cottonseed meal having a fiber content of 13.52 per cent with the following results:

TABLE 4.

*Crude fiber obtained in cottonseed meal by a delay in acid filtration.*

| DELAY OF 7 MINUTES | DELAY OF 14 MINUTES | DELAY OF 28 MINUTES |
|---|---|---|
| *per cent* | *per cent* | *per cent* |
| 13.69 | 13.71 | 13.44 |
| 13.74 | 13.63 | 13.61 |
| 13.22 | 13.57 | 13.50 |
| 13.47 | 13.47 | ..... |
| Average..13.53 | 13.60 | 13.52 |

[1] *J. Ind. Eng. Chem.*, 1915, 7: 676.

TABLE 5.

*Crude fiber obtained by a delay in alkali filtration.*

| DELAY OF 7 MINUTES | DELAY OF 14 MINUTES | DELAY OF 28 MINUTES |
|---|---|---|
| *per cent* | *per cent* | *per cent* |
| 13.49 | 13.62 | 14.16 |
| 13.30 | 13.37 | 14.00 |
| 13.04 | 13.67 | 14.14 |
| ..... | 13.95 | 14.31 |
| Average..13.28 | 13.65 | 14.15 |

In delaying acid filtration, little change is noted in the results, but in the alkali filtration with 7 minutes' delay, the results are lower, due to the additional action of the hot alkali. In the 14- and 28-minute periods, where the solutions have had a chance to cool somewhat, reprecipitation of material once dissolved occurs and the results are noticeably higher. These two samples are good examples of low and high protein feeds.

The next variation of the method studied was the effect of different types of containers upon the fiber result. The following types of containers were used and the results obtained are as follows:

TABLE 6.

*Crude fiber obtained using different containers.*

| 500 CC. ERLENMEYER FLASK | 750 CC. ERLENMEYER FLASK | 1 LITER ERLENMEYER FLASK |
|---|---|---|
| *per cent* | *per cent* | *per cent* |
| 9.41 | 9.61 | 9.23 |
| 9.55 | 9.59 | 9.52 |
| 9.38 | 9.37 | .... |
| 9.34 | ... | .... |
| Average..9.42 | 9.52 | 9.38 |

A study of these results indicates that the assay beaker and the 500 and 750 cc. Erlenmeyer flasks are satisfactory as containers. The results obtained with a 1-liter Erlenmeyer flask, however, are not satisfactory and this is due to the fact that it is practically impossible to prevent charring around the sides of the container. With only 250 cc. of solution in the flask there is not sufficient depth to prevent the sides of the flask being heated to the charring point. On the other hand, the other containers will have at least 1½ inches depth of solution in them and this is sufficient to prevent charring.

It was noted that some analysts were not removing the ether extract from the sample before determining crude fiber. This variation was studied with the following results: 9.77, 9.62, 9.68 and 9.56 per cent of

crude fiber; average, 9.66 per cent. This is to be compared with an average of 9.50 per cent obtained with the usual method.

These figures show that unless the ether extract is removed before crude fiber is determined the results will be higher, the difference depending, of course, upon the amount of fat in the sample. In order to make sure that the presence of the ether extract was increasing the fiber content, the percentage of fat in the sample was obtained and the amount of soap calculated, which would be formed from this fat during alkali digestion. Next, a fat-free charge was digested with 200 cc. of boiling sulfuric acid, filtered and just before the alkali digestion was started the calculated amount of ivory soap was added and the determination continued in the regular way. The following results were obtained: 9.61, 9.68, 9.72 and 9.60 per cent of crude fiber; average, 9.65 per cent. These results indicate that the presence of the soap has some inhibitive action which is not clearly understood but it is probable that this action is mechanical rather than chemical. Other samples were tried, including a linseed meal with a high fat content. Here the difference was very much greater, the presence of the fat making a difference of nearly 2 per cent in the fiber results.

In carrying out this work a very interesting point was noted in connection with the ether extract and it seems worth while to mention it here, although the matter does not directly concern the crude fiber determination. A charge was taken which did not have the ether extract removed. It was digested with boiling 1.25 per cent sulfuric acid for 30 minutes and washed thoroughly. The residue was then completely dried and the ether extract determined, which proved to be 5.63 per cent. This was a gain of 1 per cent over the ether extract in the original sample, which only contained 4.62 per cent. No fat was present in the substance after the alkali digestion, which shows that it was completely saponified during this digestion. The increase in the fat content caused by the acid digestion may have been due to the liberation of certain substances soluble in ether which were held in the cells and cell membranes in such a way that they were not obtained in the ordinary ether extraction.

Numerous inquiries have been made as to the effect of the degree of fineness of the material upon the fiber content. In order to more clearly understand this matter the following work was undertaken. A sample of alfalfa hay was divided into three portions. One portion was ground to pass a 20–, one a 40–, and the other a 60-mesh sieve. Each portion was mixed thoroughly and crude fiber determined with the following results:

TABLE 7.

*Crude fiber obtained on sample of alfalfa hay after grinding to different degrees of fineness.*

| 20-MESH SIEVE | 40-MESH SIEVE | 60-MESH SIEVE |
|---|---|---|
| *per cent* | *per cent* | *per cent* |
| 34.54 | 31.64 | 30.37 |
| 33.79 | 31.56 | 30.02 |
| 33.57 | ..... | ..... |
| Average. .33.97 | 31.60 | 30.20 |

From these results it is apparent that the finer a sample is ground the lower the fiber content will be. Also, better checks were obtained from the sample passed through a 40-mesh sieve and this has uniformly proved to be the case upon other work[1]. Ground alfalfa was selected because of its high fiber content and because difficulty is usually experienced in securing check results due to the different amounts of fiber in the leaves and stems and the difficulty of properly mixing these parts.

The next point of interest was the study of different filtering media. Many analysts have the idea that the chief trouble with fiber work is due to the filtering material, linen, asbestos, or glass wool, which varies in its physical characteristics and necessarily in filtering efficiency. The reports from collaborators laid great stress upon different grades and quality of linen. The following results were obtained, using linen for both filtrations and having no asbestos present: 9.27, 9.22, 9.27, 9.10 and 9.27 per cent of crude fiber; average, 9.23 per cent.

When linen was used for the first filtration and a Gooch crucible for the second, and no asbestos was present, the following results were obtained: 9.38, 9.35, 9.34, 9.28, 9.16 and 9.34 per cent of crude fiber; average, 9.31 per cent.

When linen was used for the first filtration and filter paper for the second, and no asbestos was present, the following crude fiber figures were obtained: 9.37, 9.29 and 9.24 per cent; average, 9.30 per cent.

From these results two important conclusions are drawn:

(1) Regardless of the filtering material the results are practically the same.

(2) These results are all about 0.2 per cent lower in fiber where no asbestos is used.

Some chemists have made use of 350-mesh copper gauze as a filtering medium and this was tried, both on the mixture of cornmeal and alfalfa and on the straight cottonseed meal.

Filtering after acid digestion through 350-mesh copper gauze and after alkali digestion through a prepared Gooch crucible gave the following

[1] *J. Ind. Eng. Chem.*, 1915, 7: 676.

results on a mixture of cornmeal and alfalfa: 9.48, 9.55, 9.46 and 9.58 per cent of crude fiber; average, 9.52 per cent. This is to be compared with 9.50 per cent, the average obtained with the method ordinarily employed. The following results were obtained when cottonseed meal was filtered first through a copper gauze and then through a prepared Gooch crucible: 13.85, 13.74 and 13.80 per cent of crude fiber, average, 13.80 per cent; and 13.93, 13.82 and 13.86 per cent of crude fiber, average, 13.87 per cent, when cottonseed meal was filtered first through linen and then through a prepared Gooch crucible.

The results obtained with the use of copper gauze checked surprisingly close with those obtained with linen and there seems to be no reason why 350-mesh copper gauze could not be used as well as linen. However, it was found that the gauze is very expensive and there probably would be objections to its use on this ground.

In order to make sure of the conclusions in regard to efficiency in filtering media it was necessary to ascertain whether any of the charge was passing through the linen cloths during filtration[1]. To determine this, the acid filtrate with all the washings was caught directly in a weighed Gooch crucible.

The following results were obtained from the same mixture of alfalfa and cornmeal used in previous work: 0.0050 and 0.0058 gram. This represents the amount of material which passes through the linen during filtering and washing and was caught in the Gooch crucible.

A sample consisting of a mixture of ground alfalfa and cottonseed meal was next tried and it was found that 0.0050 and 0.0075 gram passed through linen.

This same sample was filtered through the 350-mesh copper gauze with slightly higher results—0.0148 and 0.0136 gram.

A sample consisting of straight cottonseed meal was next tried, 0.0085 gram filtering through linen, and 0.0326 gram filtering through 350-mesh copper gauze.

This last result shows more of the substance coming through the wire gauze than through linen.

During this filtration work, many different grades of linen were used with practically the same result in all cases. Wire of 350-mesh allows more loss of material during filtration than linen but the writers are of the opinion that the substance which passes through the filter is mostly dissolved by the alkali and the loss, therefore, is negligible because the results with wire and cloth are found to be almost identical.

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[1] *J. Ind. Eng. Chem.*, 1910, 2: 280.

Linen should be of such character that while filtration is rapid no great amount of solid matter passes through. Many samples of linen have been tested and linen of the texture previously mentioned has always been found efficient, regardless of coarseness or fineness of weave. This is one of the most interesting points developed, for it has been regarded in the past that texture of linen was an important feature.

Work was undertaken to determine, if possible, just what effect the presence of the asbestos had upon the charge. The following results were obtained upon the mixture of cornmeal and alfalfa when asbestos was present: 9.48, 9.46, 9.55 and 9.50 per cent of crude fiber, average, 9.50 per cent; and 9.34, 9.35, 9.28 and 9.38 per cent of crude fiber, average, 9.34 per cent when no asbestos was used. This shows a difference of approximately 0.2 per cent of fiber in this type sample.

The same procedure was tried with a sample of cottonseed meal: 13.85, 13.81, 13.58 and 13.54 per cent of crude fiber, average, 13.70 per cent, was obtained when asbestos was used and 12.95, 12.97, 12.93 and 12.69 per cent of crude fiber, average, 12.89 per cent, when no asbestos was used.

The above results show a difference of 0.82 per cent of fiber for this type of sample. This may be accounted for by the much increased time of filtration when no asbestos was used.

The same variation was tried upon a sample of high fiber content, for example, alfalfa: 31.64 and 31.56 per cent of crude fiber, average 31.60 per cent, was obtained when asbestos was used and 31.38 and 31.30 per cent of crude fiber, average, 31.34 per cent, when no asbestos was used.

These results show the effect of the presence of asbestos in the fiber determination upon various types of samples of varying fiber content. The greatest difference is manifest in the cottonseed meal, where there is only about 13.50 per cent of fiber, yet the difference here is greater than for any other type of sample used.

After the fact was established that asbestos slightly raises the fiber values, the question naturally arose as to how the asbestos produced this effect. With this idea in mind, work was undertaken in which asbestos was used in every step of the determination with the following results: 9.10, 9.08, 9.07 and 9.12 per cent of crude fiber; average, 9.09 per cent.

The following results were obtained when asbestos was added after the acid digestion and before filtering the acid: 8.96, 8.96, 8.99 and 8.93 per cent of crude fiber; average, 8.96 per cent.

The loss noted is probably due to the inhibitive action of the asbestos during acid digestion, although it is not known just what this inhibitive action is[1].

[1] *British Med. J.*, 1913, 2: 1360.

When asbestos was added after filtering the acid and just before boiling with alkali the following results were obtained: 8.86, 8.84 and 8.88 per cent of crude fiber; average, 8.86 per cent.

This difference is probably due to the inhibitive action of the asbestos and the additional action of the acid, due to slowness of filtration.

Another sample was run, adding asbestos after the alkali digestion and just before alkali filtration with the following results: 8.78, 8.80 and 8.81 per cent of crude fiber; average, 8.80 per cent. The small loss noted is, of course, attributed to the inhibitive action during acid and alkali digestion and loss by slow filtration.

Asbestos was carried along in a separate container and added just before alkali filtration with the following results: 8.76 and 8.74 per cent of crude fiber; average, 8.75 per cent.

This result is almost identical with that obtained from eliminating asbestos entirely.

From the above results it is evident that the difference in fiber content, due to the presence of the asbestos, is caused mainly by the inhibitive action during digestion and, to a smaller extent, by eliminating the loss originally sustained by slowness in filtration.

Attention was next turned to the possibilities of errors during incineration. Experience has shown that crude fiber should be completely incinerated at comparatively low temperatures, especially when asbestos is carried along with the sample. The fibrous material present in feeds is of such a nature that it should be completely burned off after 20 minutes in an electric muffle at a dull red heat. Ignition at high temperatures will tend to increase the loss which is calculated as crude fiber, as the additional loss in weight is due to some action on the asbestos or volatile salts present and certainly nothing closely related to fiber[1].

## CONCLUSIONS.

After due consideration of the figures given in this work, together with the experience gained during this investigation, the writers consider the following instructions and details to be such that if carefully followed in every respect no difficulty should be experienced in getting good checks when working upon cottonseed meal or other substances by individual or different analysts.

### REAGENTS.

*Dilute sulfuric acid solution.*—Contains 1.25 grams of sulfuric acid per 100 cc.

*Dilute sodium hydroxide solution.*—Contains 1.25 grams of sodium hydroxide per 100 cc., free, or nearly free, from sodium carbonate.

The strength of these solutions must be accurately checked by titration.

*Asbestos.*—The variety found best adapted for this work was tremolite, with a refractive index of 0.1635 having fibers ranging in diameter from a maximum of 0.02–0.002 mm.

[1] *J. Ind. Eng. Chem.*, 1915, 7: 676.

This asbestos first must be digested on the steam bath overnight with 5–10% sodium hydroxide and thoroughly washed with hot water. It is next digested overnight with 5–10% hydrochloric acid and again washed thoroughly with hot water. Next, it is completely ignited in an electric muffle at bright red heat.

### APPARATUS.

*Liebig water-jacketed condenser.*

*Container.*—Any container that will give 1½ inches depth of boiling solution and allow the use of a Liebig condenser.

It has been found especially advantageous to use an iron plate between the container and flame: first, to distribute the direct heat which will prevent bumping; and second, to prevent heat from coming in contact with the flask, thereby reducing charring.

### DETERMINATION.

Extract 1–2 grams of the dry material with ordinary ether, or use the residue from the ether extract determination, and transfer the residue, together with approximately 1 gram of asbestos, to the container. Where the residue from ether extract is used and the proper amount of asbestos has already been added, further addition is unnecessary. Using a calibrated beaker, add 200 cc. of boiling dilute sulfuric acid to the contents of the container, which is immediately placed on the heating apparatus and connected with a water-cooled Liebig condenser. It is essential that the contents of the flask come to boiling within a minute after being placed upon the apparatus and that the boiling continue briskly for 30 minutes. It is found best to rotate the flask with the hand about every 5 minutes in order thoroughly to mix the charge. Care should be taken to keep the sides of the flask above the solution free from the sample. A blast of air conducted into the flask will serve to reduce the frothing of the liquid. Remove the flask at the expiration of the 30 minutes and immediately filter through linen in a fluted or ribbed funnel and wash with boiling water until the washings are no longer acid.

Next wash the charge and adhering asbestos back into the assay flask with 200 cc. of boiling dilute sodium hydroxide solution, using a 200 cc. wash bottle. By spreading out the linen on a large glass funnel (the stem of which has been removed) and using a 200 cc. wash bottle of sodium hydroxide, the transfer of the sample to the original container is very easily accomplished. Previous to this, the sodium hydroxide solution has been brought to boiling and kept at this temperature under a reflux condenser, while in use. (The sodium hydroxide solution under the reflux condenser is best transferred to a 200 cc. wash bottle by means of a bent tube through which the liquid is forced by blowing into a tube connected with the top of the condenser.) Then place the flask on the heating apparatus, connect with reflux condenser and boil for exactly 30 minutes. When running a set of fiber determinations the boiling of the alkali should be so timed that the contents of the different flasks will reach the boiling point approximately 3 minutes apart. This provides sufficient time for filtration. The last filtration takes place directly through a Gooch crucible, which has previously been prepared with a thin but close felt of ignited asbestos. Employ suction and wash the contents thoroughly with hot water and then with about 15 cc. of 95% alcohol.

Dry the crucibles with their contents to constant weight at 110° C. in an electric oven, usually overnight. After weighing, incinerate the crucibles in an electric muffle at a *dull red heat* until the carbonaceous matter has been removed, generally for 20 minutes, cool in a small, tight, efficient desiccator and weigh. The loss in weight is taken as crude fiber.

SUMMARY.

(1) Liebig condensers are the most efficient that may be used.

(2) Samples taking more than 5 minutes to filter after digestion should not be reported.

(3) Samples of high protein content, under delayed filtration, act just opposite to those of low protein content.

(4) Any container having 1½ inches of boiling depth may be used.

(5) The presence of fat in charge during fiber determinations noticeably raises the results.

(6) There is a gain in ether extract when the charge has been previously digested with 1.25 per cent sulfuric acid.

(7) The finer the material is ground the lower the fiber content. More uniform results are obtained using a 40-mesh sieve.

(8) A method capable of giving concordant results is given.

## THE DETECTION OF GROUND BRAN IN SHORTS.

By J. B. Reed (Bureau of Chemistry, Washington, D. C.).

Bran is a dairy feed and shorts is primarily a hog feed. The demand for dairy feed in the summer when cattle are out to pasture is low and since that is the season for hog raising, the demand for hog feed is high. Consequently, the price of shorts is much higher than bran at this season and sometimes it is fifteen or twenty dollars per ton higher. On the other hand, in the fall when the cattle are no longer out to pasture the demand for bran increases and at the same time the hogs are killed and marketed and the demand for shorts decreases, with the result that the prices of bran and shorts become more nearly equal. There is, then, a distinct advantage to manufacturers to run their mills so as to produce all shorts possible at certain seasons and there is a temptation to grind bran into shorts which some manufacturers have difficulty in resisting. It is therefore necessary in control work to be able to detect it.

Somewhat over a year ago the following item appeared in the Community Miller and was called to the attention of the Bureau of Chemistry by the Chief of the St. Louis Food and Drug Inspection Station:

MORE MONEY FOR YOUR BRAN.

We find that a great many mills are grinding their broad bran into shorts and are netting 20 cents more per hundred weight. There are several inexpensive machines on the market which are specially adapted for this work and they soon pay for themselves on bran alone, not to mention other grinding which they are able to do.

The practices advocated in this item would manifestly be in violation of the Food and Drugs Act and the Bureau of Chemistry immediately issued a press notice and wrote the Community Miller as follows:

THE COMMUNITY MILLER,
Chicago, Illinois.

*Gentlemen:* The Bureau's attention has been called to a short article on page 4 of the "Community Miller" for October, 1919, entitled "More Money for Your Bran". This article recommends the grinding of the bran for sale as shorts.

You may be interested to know that this practice is considered by the Bureau to be in violation of the Federal Food and Drugs Act, if the product is brought within the jurisdiction of that law. In fact, a number of actions have recently been instituted under the law, based on shipments of reground bran sold as shorts.

We assume that you were not informed in regard to the attitude of the Department in the matter, and are therefore taking this opportunity of calling it to your attention, and suggesting that you advise your readers in order that they may not unwittingly be led to violate the law.

Respectfully,
Signed—C. L. ALSBERG, *Chief.*

A copy of the press notice was also sent to them. The following item, which includes the press notice, appeared in the next issue of the Community Miller:

MISBRANDING.

In a recent issue we published a short article which had been submitted to us, relative to how to realize 20c. per cwt. by grinding bran and mixing with middlings. We wish to call our readers' attention to the following announcement from the Department of Agriculture and caution community millers regarding wrong labeling or misrepresentation:

Seizures of shipments of stock feeds on the charge of adulteration and misbranding, because of the sale of reground bran and screenings as shorts, have been made upon the recommendation of officials of the Bureau of Chemistry, United States Department of Agriculture, who are charged with the enforcement of the Federal Food and Drugs Act.

The feed known to the trade as "shorts" contains more nutritive material than ground bran and screenings, and sells in the market for a considerably higher price. The sale of ground bran and screenings as shorts in the opinion of the officials, is not only a fraud upon the purchaser, but is demoralizing to the feed industry. Honest feed manufacturers who correctly label their feeds are placed at a disadvantage in competing with manufacturers and dealers who put out cheaper products under the names of higher priced ones.

The shipment into interstate commerce of ground bran and screenings, labeled as shorts, constitutes both adulteration and misbranding under the terms of the Federal Food and Drugs Act. Inspectors have been instructed to watch for interstate shipments of adulterated and misbranded stock feeds. Action will be taken, say the officials, in all cases found to be in violation of the law.

The matter of issuing a press notice informing the public that in the opinion of the Bureau the practice was in violation of the Food and Drugs Act was a simple matter, but the problem of detecting the presence of ground bran in shorts was something for the scientists of the Bureau of Chemistry to solve. D. B. Bisbee of the St. Louis Food and Drug Inspection Station was already working on the problem and the

Cattle Food Laboratory was asked to study it also. The result was that two entirely different methods were developed, one by Bisbee and one by the Cattle Food Laboratory.

The wheat berry contains a germ and in the regular process of milling wheat the germ necessarily finds its way into the shorts. It was obvious that if a means of separating the germ from the shorts could be found the solution of the problem would be well under way. Fortunately, the means of separation was at hand in the form of an apparatus devised by the Seed Laboratory of the Department of Agriculture for the separation of light seeds from the heavier ones. In this apparatus, use is made of a blast of air passing up through bolting cloth. By careful manipulation of the air pressure it is possible to make desired separations of lighter from heavier materials.

G. P. Walton of the Cattle Food Laboratory had an apparatus of this kind made and was using it in making separations of seed from lighter material when this problem arose. The apparatus consists of a tube about 1¼ inches in diameter and about 13 inches long, constricted somewhat at each end. The bottom is covered with a piece of bolting cloth and is inserted in a rubber stopper. It is then placed in an ordinary suction flask and the flask connected with the blast through a drying flask. At the top of the tube a gooseneck, with a flaring orifice, is inserted and a suitable receptacle is placed under the orifice.

In order to see if the germ could be separated from the rest of the shorts by means of this apparatus, a charge of shorts was introduced into the tube and the air turned on. It was found that by proper manipulation a residue was obtained which contained a considerable amount of germ but there was also a large amount of the larger bran particles and pieces of endosperm present and the separation was not at all satisfactory. The sample was then ground to pass through a 20-mesh sieve and it was found that by proper manipulation a fairly satisfactory separation of the germ could be accomplished, sufficiently satisfactory, in fact, so that the addition of any considerable amount of ground bran could be detected in a sample of shorts containing no ground screenings. The germ residue seemed to run from 4 to 6 per cent on authentic samples of shorts.

The addition of so-called mill run of ground screenings complicated matters. The small particles of grains and weed seeds weigh as much or more than the germ particles and, consequently, they remain behind with the germ. However, with experience acquired by running a great number of known samples of shorts it is possible for one to judge fairly accurately from the amount of germ present in the residue as to whether or not the shorts contain ground bran. If the residue is less than 3 per cent the suspicion is aroused that there is ground bran present or that the mill is being manipulated. In these cases, an inspector is sent to the mill to ascertain what is taking place. If the residue is less than 2 per cent, it

is pretty certain that the product consists in whole or in part of ground bran.

However, even if the residue is found to be greater than 3 per cent, it is not necessarily concluded that no ground bran is present. If, in the analyst's judgment, the residue contains very little germ and is largely screenings, the conclusion is reached that the product contains ground bran even if 6 or 8 per cent residue is obtained, but these are extreme cases and only those who have had a great deal of experience would be in a position to judge. B. H. Silberberg of the Microchemical Laboratory has followed this work very closely and has assisted in making most of the determinations. Her results agree very closely with those of the writer, and it seems probable that others with sufficient experience would agree closely in most cases.

The details in carrying out this determination are as follows:

Grind the sample to pass a 20-mesh sieve. Make the determination with 10 grams of the material but, owing to the fact that the apparatus will not handle 10 grams very satisfactorily, weigh out two 5-gram samples. Introduce the first 5-gram sample into the apparatus, turn on the blast gradually and make a rough separation but leave sufficient residue to be certain that none of the germ is carried over. Then remove the residue, add the second 5 grams and repeat the process. Then replace the residue from the first 5 grams and place a clean receptacle under the gooseneck. Clean the whole residue by repeating the blasting process until satisfied that all other material possible is separated from the germ.

In carrying out the test, the flour will pass over first and an idea may be obtained as to about how much is present. The fact that a sample contains very little flour does not necessarily indicate that the product is not shorts. In fact, the largest and most efficient mills produce shorts with very little flour, while shorts from smaller, less efficient mills often contain considerable flour. It would naturally be expected that shorts and bran from the more efficient mills would contain less flour, since the production of flour is the object of milling wheat.

The fact that bran from the smaller, less efficient mills contains considerable flour and endosperm particles brought up a very interesting question in connection with the matter of ground bran in shorts. It was found that several concerns which do not mill wheat were putting shorts on the market. After the press notice, previously mentioned, was issued representatives of these concerns came to the Bureau of Chemistry to find out where they stood. They put up a very plausible argument. They claimed that they were buying bran from the smaller, less efficient mills which contained a considerable amount of shorts material and they were merely doing to it that which the larger, efficient mills do to the bran in their regular process; that is, subjecting it to more attrition with the result that they were actually producing shorts. There seemed to be something in their argument and they submitted some samples

which seemed to pass the test for shorts. However, when the Bureau of Chemistry picked up samples of the products of these concerns they did not pass the test. They were not doing what they claimed; that is, subjecting the bran from the smaller mills to the same treatment that it receives at the larger mills, but were actually grinding the bran. The Bureau of Chemistry conducted a vigorous campaign against the practice of grinding bran to make shorts, with the result that it is having very little difficulty along these lines at present. It might be of interest to say that the inspectors found Williams or Greundler grinders in a good many flour mills inspected but in most cases they had not been installed. It is not known whether the millers had taken their cue from the item in the Community Miller or whether the Community Miller got the idea from the fact that some mills were doing it but probably the latter is the case; at any rate, the activity of the Bureau of Chemistry seemed to have discouraged the practice so that they did not install the grinders.

The method developed by Bisbee, page 75, is entirely different from the one just described. However, the reports on a large number of samples examined by the two different methods and reported independently agreed surprisingly well.

## THE DETECTION OF THE ADULTERATION OF SHORTS.

By D. B. Bisbee (U. S. Food and Drug Inspection Station, U. S. Custom House, St. Louis, Mo.).

Shorts is a term applied to a mixture of particles of bran, germ and endosperm, all derived from wheat during its milling into flour. As frequently marketed, shorts contain low-grade flour and particles of ground weed seeds and other screenings.

Attention is called to the following important factors in the detection of the adulteration of shorts:

(1) Shorts are produced only by flour mills. Any mill producing shorts and not flour is producing false shorts.

(2) In a flour mill, the bran is tailed off over a wire screen of 18 to 22 mesh. A very few mills use a finer screen, some as fine as 32 mesh. It is admitted by all millers that an 18-mesh screen is as coarse as can be used. All particles in true shorts are, therefore, not above 18 mesh in size.

(3) The better and more complete the separating machinery in a mill, the more complete the recovery of flour and the "leaner" the shorts. A small mill (1000 barrel or less daily capacity) lacking the extensive and expensive separating machinery which is found in a very large mill, produces the richer shorts.

(4) The large mills usually market three grades of shorts—brown, white and gray. Some mills separate the germ, not selling it as a part of

the shorts; other mills separate a part of the coarse middlings, as they are passing through the mill, selling this separated part as breakfast food.

(5) As a corollary to (3) and (4), it must be remembered that the brown shorts produced by a very large mill, such as the Pillsbury mill, may be so completely freed from germ and endosperm particles that in nearly all respects it resembles a reground bran.

From these five facts, it is seen that it is necessary to know the source of a lot of shorts before accurate judgment can be passed upon it. Is this source a flour mill? If so, what is its daily capacity? Does it market more than one grade of shorts? Are the middlings streams scalped to produce breakfast food or other by-products? With correct answers to these questions and with data obtained as described below an accurate judgment may be formed as to whether a sample of shorts has been adulterated.

The writer has recognized the following as adulterants of shorts: (1) Coarse bran; (2) reground bran; (3) ground rice hulls; and (4), flour (if this be termed an adulterant). He has found, substituted entirely for shorts: (1) Reground bran with ground screenings; and (2), a mixture of reground bran and flour. The writer first separates the sample of shorts into portions by means of wire screens of 40, 60 and 100 mesh. If it is evident that the sample contains coarse bran, a 20-mesh screen is first used. The sample is thus divided into what is termed the 20-mesh portion (that part retained on a 20-mesh wire screen), the 40-mesh portion, the 60-mesh portion, the 100-mesh portion, and the fines. Ash is then determined in the original sample. Fiber is determined in the original sample and in each portion except the fines. Each portion is examined with a strong hand lens, magnifying ten times. With these data and with the necessary information as to the producing mill, the purity of the product may be judged.

The ash in bran is 6 per cent or more; in shorts, from 5.5 down to as low as 2 per cent, depending upon the grade; the richer the shorts, the lower the ash. Fiber in bran will run about 11 per cent; in all true shorts examined—save the Pillsbury and the Washburn-Crosby brown shorts—fiber did not exceed 8.5 per cent, usually running less than 8 per cent; the richer the shorts the lower the fiber. The fiber in the various portions obtained by screening will vary with the proportion of bran particles.

The examination of the various portions by means of a strong lens will reveal the ingredients of these portions. The 20-mesh portion is entirely bran. If a true shorts is under examination, the 40-mesh portion is almost entirely bran, but contains a few germ and endosperm particles. The 60-mesh portion is chiefly bran, but contains very many germ and endosperm particles. The 100-mesh portion consists of germ and endo-

sperm particles with considerable bran. The fines contains some fine bran particles, but is almost entirely endosperm particles.

How do the various adulterants affect these analytical results?

Coarse bran is entirely separated in the 20-mesh portion and is easily recognized. It raises the ash and fiber content of the original sample.

Reground bran raises the ash and fiber content of the original sample and of each portion. If reground bran has been entirely substituted for shorts, each portion will be seen to consist almost solely of bran particles and will have a fiber content of 9 to 11 per cent. As some of the endosperm always adheres to the bran (more to soft wheat bran than to hard wheat bran), and as this endosperm material is separated from the bran and partly pulverized when the bran is reground, the 60- and 100-mesh portions and the fines will contain a small proportion of endosperm particles.

A mixture of reground bran and flour will have a fiber and ash content normal to true shorts, but the screens at once reveal this form of adulteration, as all of the flour passes all screens and can be recognized easily in the fines, while the various portions are seen to be similar to the portions obtained by screening a reground bran.

Ground rice hulls are very high in fiber and ash. The original sample and the various portions will be found to contain startling amounts of fiber. In the 40-mesh portion of one sample the writer found 17 per cent of fiber. Rice hulls are easily recognized by decanting the light bran fiber from the heavy rice hull fiber (after the alkaline fiber digestion), the rice hull particles retaining their form and characteristic surface markings and having a lemon color.

When weed seeds are ground into shorts, their seed coats will be recognized by the eye. The particles of their endosperms will be seen in the 60- and 100-mesh portions and allowance must be made for them in judging the shorts, as they lower the fiber and ash in proportion to their amount.

Flour, when mixed with shorts, lowers the ash and fiber of the original sample, but has no effect upon the various portions, as it passes all screens. It may be recognized in the fines by the lens and by its smooth, slick feeling.

Before passing judgment upon shorts samples, the analyst should obtain many samples of true shorts of various grades and also several samples of known adulterated shorts. These known samples should be examined together with and in comparison with the shorts under investigation.

It is particularly true with this method that experience is necessary before the analyst can feel confidence in his judgment. Should there be any doubt in his mind, an inspection of the producing mill should be made and known authentic samples of its shorts examined in comparison with the suspected sample.

# REPORT ON STOCK FEED ADULTERATION.

By B. H. SILBERBERG (Bureau of Chemistry, Washington, D. C.), *Associate Referee.*

Since the question of the quantitative determination of ingredients of mixed feeds still appears to be a live one, it was assumed that it would be of interest to continue the work along this line, confining it to a mixture of two ingredients only. Many feeds, particularly by-product feeds, are in themselves rather complex, containing two or more ingredients; *e. g.*, hominy feed contains corn bran, corn starch, some horny endosperm of the corn and, if properly made, corn germ or corn germ meal; also oat feed contains oat hulls, oat bran tissues and oat starch. Commercial rice bran is another such by-product feed containing normally rice bran tissues, more or less broken rice which appears microscopically as starch, and not more than 10 per cent of rice hulls. The commonest form of adulteration of rice bran is an excess of rice hulls. Your associate referee devised a method for the quantitative determination of rice hulls in rice bran. This method gave results which correlated so closely with those obtained chemically, that your associate referee thought it timely to submit the method to the collaborators. The method is as follows:

Thoroughly mix the sample to be examined. Draw out a small portion and grind until it all passes through a 60-mesh sieve. Weigh 4 mg. on a slide ruled in parallel lines $\frac{1}{20}$ inch apart, or transfer to the ruled slide after weighing. Add just sufficient chloral hydrate solution (1 to 1) to fill in under the cover glass, which should be preferably square (about 22 mm.). After the cover glass is in place warm gently, but do not boil. This is to eliminate the starch masses and clear the tissues.

Use a magnification of about 90 diameters (6 compensating ocular and 16 mm. apochromatic objective). Count the particles of hull tissue. The high refraction and yellowish green color of the hull particles will aid in distinguishing the small pieces in which the structure is not easily recognizable. In order to avoid duplicate counting, it is well to disregard those particles which extend over the upper line of the strip.

From results obtained on standards containing known amounts of hulls, the approximate amount of hulls present in the sample can be estimated.

The collaborators received four reference samples of rice bran containing 10, 15, 20 and 25 per cent respectively of rice hulls, a sample of ground rice bran free from hulls, one of ground rice hulls, two samples, A and B, containing percentages of rice hulls known only to the associate referee, and a ruled slide to be used in making counts. They were advised to familiarize themselves with the microscopic appearance of rice hulls and rice bran, and then to count at least two, preferably four or five slides, of each standard or reference sample and of each unknown. The system of counting each standard twice, each unknown twice, and then repeating the count on the standard nearest to the unknown, was recommended; also that this operation be repeated before making a report.

It was requested that all counts be reported, on standards as well as unknowns, with conclusions as to the amount of hulls in the unknowns. This was complied with in all but two cases in which no data were given except the conclusions, making it impossible to investigate the reason for the results being so far from correct in one of these cases. It was stated in this instance that the analyst was somewhat inexperienced in microscopic work, although this same statement was made in the case of another analyst whose results were correct within 0.1 and 0.2 per cent.

The counts obtained by most of the collaborators on the reference samples indicate that only the particles of epidermal tissue were counted instead of all the particles of hull as directed. But the counts thus obtained appear to be so consistent and the results so good it seems advisable to use the number of epidermal particles rather than all the hull particles as a factor and change the method to read "Count the particles of epidermal tissue of the hull" instead of "Count the particles of hull tissue".

RECOMMENDATION.

The results as shown in the table, meagre as they are, appear to be of such character as to warrant the recommendation that the method be adopted as a tentative method for the quantitative microscopic determination of rice hulls in rice bran, and that it be made the subject of further study.

*Counts obtained on standards and unknowns.*

| ANALYST | AVERAGE COUNTS ON STANDARDS | | | | REPORTS ON UNKNOWNS | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | Sample A 12 per cent | | Sample B 18 per cent | |
| | 10 per cent | 15 per cent | 20 per cent | 25 per cent | Average count | Reported | Average count | Reported |
| | | | | | | *per cent* | | *per cent* |
| 1 | 103 | 162 | 196 | ... | 127 | 12.2 | 185 | 17.9 |
| 2 | ... | ... | ... | ... | ... | 20.0 | ... | 24.2 |
| 3 | 97 | 142 | 180 | 220 | 101 | 10 | 147 | 15 |
| 4 | 92 | 162 | 202 | 251 | 134 | 13.3 | 173 | 17.1 |
| 5 | 195 | 288 | 386 | 514 | 271 | 13–14 | 437 | 22 |
| 6 | 226 | 325 | 397 | 554 | ... | ... | ... | ... |
| 7 | ... | ... | ... | ... | ... | 10+ | ... | 15+ |

## REPORT ON SACCHARINE PRODUCTS.

By H. S. PAINE (Bureau of Chemistry, Washington, D. C.), *Referee.*

On account of delay in being advised of his appointment, the Referee on Saccharine Products is unable to report any progress with respect to work actually accomplished. Associate referees have been appointed on

the following subjects: sugar-house products; honey; and maltose products. J. F. Snell was unable to serve again as Associate Referee on Maple Products and, in view of the short time remaining before the meeting of this association, it was impossible to secure any one to succeed him. Because of the impossibility of doing any work in the time available, each associate referee was requested to present to the association at this meeting a tentative program of work for the coming year. If work is started immediately on the programs outlined it should be possible to secure results of value during 1921 and the remainder of 1920.

No report on honey was made by the associate referee.

No associate referee on maple products was appointed and no special report on this subject was presented.

No report on maltose products was made by the associate referee.

No report on sugar-house products was made by the associate referee.

The appointment of the following committees was announced by the president:

*Committee on auditing:* A. J. Patten of Michigan and H. H. Hanson of Delaware.

*Committee on nominations:* R. N. Brackett of South Carolina, C. H. Jones of Vermont and W. W. Skinner of Washington, D. C.

*Committee on resolutions:* William Frear of Pennsylvania, Julius Hortvet of Minnesota and E. W. Magruder of Virginia.

*Committee to wait upon the Secretary of Agriculture:* B. B. Ross of Alabama and C. H. Jones of Vermont.

*Committee to wait upon the Honorary President:* W. F. Hand of Mississippi and A. J. Patten of Michigan.

The meeting adjourned at 1 p. m. to reconvene at 2 p. m.

## FIRST DAY.

# MONDAY—AFTERNOON SESSION.

## REPORT ON FERTILIZERS.

By R. N. BRACKETT (Clemson Agricultural College, Clemson College, S. C.), *Referee.*

Upon my belated arrival at the 1919 meeting of the association, I found that in addition to the General Referee on Fertilizers, the association had appointed the usual associate referees.

I persuaded W. H. Ross to act as Chief Associate Referee on Borax, on account of the work which he had already done on this subject and because I felt that he would take a special interest in the work. In addition, I appointed R. B. Deemer, G. F. Lipscomb and C. A. Butt to serve as associate referees in working out a method or methods of determining borax in fertilizers and fertilizer materials.

Only two new subjects have been brought up for investigation this year: the working out of a suitable method for the determination of borax in fertilizers and fertilizer materials; and a special study of a suitable method for the analysis of precipitated superphosphate. There has been quite a considerable amount of work done upon the former and a lively interest taken in the investigation. The following methods have been suggested and worked out with considerable care:

*Qualitative methods.*—Methods by Richardson, Pope and Ross, and Rudnick, all involving the use of turmeric or curcumin. It has been suggested that these methods, to some extent at least, may be used quantitatively.

*Quantitative methods.*—(1) Distillation methods by Richardson, Bartlett, and Carpenter and Magruder and Breckenridge. (2) Methods without distillation by Ross-Deemer, Lipscomb-Inman-Watkins, and by Jones of Vermont. Some of these methods were published[1] in order that they might be tried out by as many analysts as possible before this meeting.

## REPORT ON THE DETERMINATION OF BORAX IN FERTILIZERS AND FERTILIZER MATERIALS.

By WILLIAM H. ROSS (Bureau of Soils, Washington, D. C.), *Associate Referee.*

The different methods that have been proposed for the determination of borax may be classed conveniently into two groups as represented by

[1] *Am. Fertilizer*, 1919, 51: (No. 13), 66; *Ibid.*, 1920, 52: (No. 5), 57.

the Gladding[1] and the Thompson[2] methods. In the methods of the first group the boric acid is separated from the constituents with which it is associated by distilling with methyl alcohol, and then determined in the distillate in which it is recovered by titrating with standard alkali solution. In the methods of the second group, the principle of the procedure is reversed. The boric acid is not volatilized but the interfering substances are removed instead by precipitation and the boric acid in the final filtrate is then determined by titration in the usual way.

When the necessity arose for determining small amounts of borax in mixed fertilizers it was found that the volatile and soluble organic constituents in fertilizers so interfered with the determination of the borax that no satisfactory results could be obtained when following the directions as outlined in the methods of either group. A number of modifications were accordingly suggested by different workers in this field, and the purpose of the present report is to give a summary of the results obtained in a comparative study of the relative merits of three of these modified methods which were selected as representative of those which have come to our attention. These methods were developed in the chemical laboratories of Clemson College, the Bureau of Soils and Swift and Co. For convenience they are designated as the Lipscomb-Inman-Watkins[3], the Ross-Deemer[4] and the Richardson distillation methods.

## PREPARATION OF SAMPLES FOR COLLABORATIVE WORK.

The samples submitted to the different collaborators consisted of four mixed fertilizer samples and three of potash salts. Sample No. 1 was a 5-10-0 fertilizer kindly supplied by J. E. Breckenridge of the American Agricultural Chemical Co. and supposedly borax-free. All qualitative tests indicated that the borax present, if any, did not exceed 0.01 per cent, and no borax was added. Sample No. 2 was the same as No. 1 with sufficient borax and potassium chloride added to approximate a 5-10-3 fertilizer containing 0.10 per cent of borax. Sample No. 3 was a 5-8-5 fertilizer prepared in the Department of Agriculture from cottonseed meal, tankage, cyanamide, ammonium sulfate, acid phosphate, potassium chloride, sand and sufficient borax to make 0.10 per cent. Sample No. 4 was the same as No. 3 with 0.75 per cent of borax added. Sample No. 5 was a Chilean nitrate of potash containing an unknown amount of borax. The results obtained by the writer in the analysis of this sample varied from 0.71 to 0.73 per cent. Sample No. 6 was the same as No. 5 with sufficient potash alum added to reduce the borax content to 0.64 per cent, assuming it to be originally 0.72 per cent. Borax

[1] *J. Am. Chem. Soc.*, 1898, **20**: 288.
[2] *J. Soc. Chem. Ind.*, 1893, **12**: 432.
[3] *Am. Fertilizer*, 1920, **52**: (No. 5), 57.
[4] *Ibid.*, (No. 6), 62.

was also added to the extent of 0.75 per cent of the final mixture, making a total of 1.39 per cent. Sample No. 6 was a mixture of potassium chloride, sodium carbonate and potassium phosphate with sufficient borax to amount to 1.00 per cent of the mixture.

The borax used was prepared by adding a known amount of boric acid of a high degree of purity to a solution of an equivalent amount of pure sodium carbonate. The solution was then evaporated on a water bath, dried at 105°C., weighed and ground to pass a 175-mesh sieve. Knowing the weight of the boric acid taken and of the product finally obtained, it could then be calculated how much of the product would have to be added to a fertilizer to give a boron content equivalent to any desired percentage of anhydrous borax. The mixed fertilizer samples were ground to 40 mesh and the mineral salts to 100 mesh. Uniform distribution of the borax through each sample was insured by thorough mixing in a mixing machine.

## INSTRUCTIONS TO COLLABORATORS.

A detailed account of each method was forwarded with the samples to each collaborator, and it was requested that each analyst submit a report on completing the analysis indicating the method which in his judgment gave most accurate results and which was considered most rapid if applied in routine analysis. No indication was given of the composition of the samples other than that four were of mixed fertilizers and three of potash salts.

Directions were also submitted for carrying out two qualitative tests for borax which it was suggested might be applied quantitatively in the case of the mixed fertilizer samples low in borax. These methods which are designated the Richardson, and the Pope-Ross[1] qualitative methods are modifications of the well-known turmeric paper and the tincture of turmeric tests. The results reported are summarized in Tables 1 and 2.

[1] *Am. Fertilizer*, 1920, 52: (No. 6), 65.

TABLE 1.

*Determination of borax in mixed fertilizers and fertilizer materials.*

| ANALYST | BORAX FOUND EXPRESSED AS ANHYDROUS BORAX | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Sample No. 1* | | | Sample No 2† | | | Sample No. 3† | | | Sample No. 4‡ | | | Sample No. 5§ | | | Sample No. 6** | | | Sample No. 7†† | | |
| | Lipscomb-Inman-Watkins | Ross-Deemer | Richardson distillation | Lipscomb-Inman-Watkins | Ross-Deemer | Richardson distillation | Lipscomb-Inman-Watkins | Ross-Deemer | Richardson distillation | Lipscomb-Inman-Watkins | Ross-Deemer | Richardson distillation | Lipscomb-Inman-Watkins | Ross-Deemer | Richardson distillation | Lipscomb-Inman-Watkins | Ross-Deemer | Richardson distillation | Lipscomb-Inman-Watkins | Ross-Deemer | Richardson distillation |
| | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* |
| A. E. King, Swift & Co., Chicago, Ill. | 0.02 | 0.03 | 0.01 | 0.08 | 0.08 | 0.10 | 0.08 | 0.08 | 0.09 | 0.60 | 0.61 | 0.67 | 0.87 | 0.81 | 0.77 | 1.46 | 1.30 | 1.26 | 1.06 | 1.00 | 0.96 |
| C. A. Butt, International Agricultural Corporation, Atlanta, Ga. | 0.00 | 0.03 | 0.02 | 0.09 | 0.07 | 0.04 | 0.10 | 0.08 | 0.07 | 0.68 | 0.85 | 0.15 | 0.74 | 0.72 | 0.62 | 1.53 | 1.52 | 1.25 | 0.99 | 0.96 | 0.87 |
| G. J. Kuhlman, jr., State Department of Agriculture, Harrisburg, Pa. | 0.04 | 0.04 | 0.03 | 0.09 | 0.08 | 0.08 | 0.09 | 0.11 | 0.12 | 1.59 | 1.21 | 1.26 | 0.96 | 1.03 | 1.04 | 2.19 | 1.50 | 1.13 | 1.18 | 0.98 | 1.01 |
| H. R. Laub and R. M. Jones, State College, State College, Pa. | 0.36 | 0.11 | 0.06 | 0.39 | 0.13 | 0.05 | 0.58 | 0.14 | 0.05 | 0.82 | 1.40 | 0.13 | 0.88 | 0.90 | 0.46 | 1.82 | 1.53 | 0.38 | 1.95 | 0.98 | 0.95 |
| J. S. Watkins, Clemson Agricultural College, Clemson College, S. C. | . | . | | 0.09 | 0.09 | . | 0.20 | 0.24 | | 0.59 | 0.53 | .... | ... | .... | .... | 1.56 | 1.48 | .... | 0.94 | 0.93 | .... |
| L. H. Crudden, Agricultural Experiment Station, LaFayette, Ind. | 0.24 | 0.03 | | 0.27 | 0.10 | . | 0.22 | 0.10 | . | 1.18 | 1.16 | | 1.12 | 1.07 | .... | 2.22 | 2.28 | .... | 1.20 | 1.25 | .... |
| R. D. Caldwell, Armour Fertilizer Works, Atlanta, Ga. | 0.76 | 0.58 | . | 0.49 | 0.50 | .. | 0.39 | 0.09 | .. | 0.65 | 0.87 | .... | 0.75 | 0.77 | .... | 1.86 | 1.74 | ... | 1.38 | 1.43 | .... |
| W. J. Gascoyne, Gascoyne & Co., Baltimore, Md | ... | 0.02 | .. | .. | 0.08 | . | . | 0.10 | .. | .. | 0.74 | .... | ... | 0.73 | .... | .... | 1.42 | .... | .... | 0.92 | .... |

* No borax present.
† Borax present, 0.10 per cent.
‡ Borax present, 0.75 per cent.
§ Borax present, 0.72 per cent
** Borax present, 1.39 per cent.
†† Borax present, 1.00 per cent.

TABLE 2.

*Detection of borax in mixed fertilizers.*

| ANALYST | BORAX ESTIMATED BY TEST | | | | | |
|---|---|---|---|---|---|---|
| | Sample No. 1 * | | Sample No. 2 † | | Sample No. 3 † | |
| | Richardson | Pope-Ross | Richardson | Pope-Ross | Richardson | Pope-Ross |
| | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* |
| A. E. King | none | none | more than 0.05 | less than 0.10 | more than 0.05 | less than 0.10 |
| C. A. Butt | none | none | less than 0.10 | less than 0.10 | less than 0.10 | less than 0.10 |
| G. J. Kuhlman, jr. | trace | trace | less than 0.10 | 0.10 | 0.10 | 0.10 |
| H. R. Laub and R. M. Jones | present | 0.10 | present | 0.10 | present | 0.10 |

* No borax present.
† Borax present, 0.10 per cent.

## COMMENTS BY COLLABORATORS.

*C. A. Butt.*—The Ross-Deemer method is most suited as regards both accuracy and speed for mixed fertilizers and mineral salts. The Lipscomb-Inman-Watkins method gives solutions which are more difficult to boil, due to bumping, and which filter much more slowly than by the Ross-Deemer method. The accuracy of the former method is impaired by the fact that an aliquot corresponding to only 1 gram of sample is recommended. In the Richardson distillation method, water-insoluble borates are likely to be formed on the addition of alkalies to mixed fertilizers and evaporation to dryness. Following this method as outlined and taking up with water after igniting, only a portion of the borax is obtained.

The Pope-Ross qualitative method was found to be much more rapid than the Richardson method and due to inability to secure all of the borax in solution by the latter method, as mentioned in the criticism of the distillation method, the Pope-Ross method is regarded as more accurate. This method gave results that enabled us to easily distinguish between materials containing more or less than 0.10 per cent.

*G. J. Kuhlman, jr.*—The Richardson qualitative method requires less cost, less work, and can stand overnight ready for the next day. The Pope-Ross qualitative method is costly but accurate. The Richardson quantitative distillation method requires too much care and borax is liable to be lost during the process of manipulation.

*H. R. Laub and R. M. Jones.*—The Ross-Deemer method is the best although not quite so fast as the Richardson distillation method which, however, gives low results.

*L. H. Crudden.*—The limewater is not so effective for precipitating the sulfates and phosphates as the barium chloride; this explains the fact that Samples Nos. 1, 2 and 3 with a low per cent of borax gave higher results with the Lipscomb method than with the Ross-Deemer method, the end point being somewhat indefinite in the former method.

*W. J. Gascoyne.*—The Ross-Deemer method is the only method by which satisfactory results could be obtained.

## DISCUSSION.

Reports have been received from eight collaborators. Four of these reports gave results with the Richardson distillation method; seven with the Lipscomb-Inman-Watkins method; and all eight with the Ross-Deemer method. So far as known, six of the collaborators had no previous experience with any of the methods. Five out of the six placed the Ross-Deemer method first as regards accuracy and one made no comment. Four out of the six also placed this method first as regards rapidity; one placed the Richardson distillation method first as regards rapidity but the results which this collaborator reported by this method were too low; and one made no comment. The remaining two collaborators reported most favorably on the respective methods with which they were most familiar but good results were also reported by the Ross-Deemer method and one of these collaborators placed this method first when phosphates are absent.

The reports show that borax in fertilizers, even when present in relatively small amounts, can be determined with a high degree of accuracy by all three methods in the hands of experienced analysts. The collaborators who had no previous experience with the Richardson distillation method as a rule reported low results with this method, while high and low results were reported with the precipitation methods.

Since this work was completed, a modification of the distillation method has been proposed by J. M. Bartlett of the Maine Agricultural Experiment Station and it is possible that this method may prove superior to any of the others in the analysis of certain classes of materials as, for example, those which are high in soluble phosphates and low in borax and which are, therefore, most difficult to handle by the precipitation methods.

Reports on the qualitative methods were received from four of the collaborators. One made no comments on either method; three placed the Pope-Ross test first as regards accuracy; two also placed this test first as regards rapidity; and one considered the Richardson test more rapid.

## RECOMMENDATIONS.

It is recommended—

(1) That the Ross-Deemer method for the determination of borax in fertilizer materials and mixed fertilizers be adopted as a tentative method.

(2) That further work be done on the comparison of the proposed tentative method with the distillation method of Bartlett.

(3) That substances other than fertilizer materials be included in the study of these methods.

# REPORT ON BORAX IN MIXED FERTILIZERS.

By R. B. DEEMER (Soil-Fertility Investigations, Washington, D. C.), *Associate Referee.*

Previous to the work upon the method which has been proposed as tentative for the determination of borax in mixed fertilizers, many attempts were made to use the distillation method developed by Chapin[1]. The results which were first obtained were very unsatisfactory as considerable difficulty was experienced in determining the point at which the volatilization of methyl borate was complete. Even after the distillation of as many as 400 cc. of alcohol, titrations of a few tenths of a cc. of 0.1N alkali were obtained. In these first trials from 5 to 10 cc. of concentrated hydrochloric acid were used in acidifying the sample to liberate the boric acid, and it was not until later that the publication of Allen and Zies[2] was reviewed. They observed that arsenic in the presence of more than a slight excess of acid is volatilized with the methyl borate and later obscures the end point when the boric acid is titrated. This suggested the use of a smaller amount of acid and, when a slight excess of acid of known amount was tried, more concordant results were obtained. Owing to the pressure of other work, the cause of this difficulty experienced with mixed fertilizers has not been investigated. However, it was further found necessary to introduce a modification of the treatment of the distillate, after the removal of the alcohol, before entirely satisfactory results were forthcoming.

The results reported by the previous investigators of this method were obtained upon much larger quantities of boric acid and boric oxide than are at present important to the association. It was thought, therefore, that results with smaller amounts of boron, and a few suggestions upon the method, might prove of interest. Chapin's article should be consulted for full details of manipulation and a discussion of the more important points of the reaction involved. The investigation reported by Allen and Zies is a very complete study of Chapin's method and contains excellent data which definitely clear up many of the points raised by him, which adds materially to the value of the method. In order to make the modifications and suggestions clear the following brief outline of the method is given:

## METHOD.

Two grams of the sample are placed in a 150 cc. side-neck distillation flask (which is referred to as the "decomposition flask"), 8 cc. of water are then added, followed by 1 cc. of concentrated hydrochloric acid and 15 grams of granular, anhydrous calcium chloride. The distillation of the methyl alcohol is started and continued until about

[1] *J. Am. Chem. Soc.*, 1908, 30: 1691.
[2] *J. Am. Ceram. Soc.*, 1918, 1: 739.

10 cc. have condensed in the decomposition flask, after which the latter is heated just enough to prevent further condensation. Distillation is continued until 150 cc. of the distillate are collected to which are now added approximately 30 cc. of 0.1N sodium hydroxide and the alcohol recovered by distillation. Ten cc. of a 10% solution of barium chloride and a few crystals of barium hydroxide are added and the solution boiled a short time to remove the last traces of methyl alcohol. The precipitate which forms is filtered off and to the filtrate is added 2–3 drops of methyl red and the solution made acid with a very slight excess of 0.5N hydrochloric acid. Carbon dioxide is then removed in the manner suggested by Chapin, and the titration of the boric acid made with 0.05N sodium hydroxide in the usual way.

## DISCUSSION.

In place of the Erlenmeyer flask which Chapin suggests it was found more convenient to use a wide-neck, 250 cc. flask for a receiver, and also to omit the water bath for heating the decomposition flask, using a direct flame instead. The adjustment of the heat applied to this flask is essential as it should not be such as to cause a reduction in the volume of its contents as the volatilization of the methyl borate should be accomplished by the vapors of methyl alcohol from the large distillation flask. If the apparatus is set up in a vertical position and a short spiral condenser substituted for the one suggested in the original method, a more satisfactory arrangement of the various parts may be made.

The composition of the precipitate formed by the addition of barium chloride to the distillate has not been investigated; however, unless this procedure is followed it is necessary, after removal of the alcohol, to evaporate the solution to dryness and ignite the residue. Chapin's discussion of the end points should be noted in particular as these are never so sharp in this method as those which are obtained in the one proposed as tentative. When neutralizing with sodium hydroxide it will be noted that the red color of the methyl red fades very gradually and it is essential that the full bright yellow of this indicator should be produced at this point. The brownish tinge noted by Chapin in the final titration marks the end of the reaction in the presence of the two indicators. In this connection it is well to remember that the volume of the solution should be below 100 cc. and, in fact, better end points can be obtained with a volume of from 50 to 75 cc. The work of Kahlenberg and Schreiner[1] explains very clearly the reason for this. A blank should be run upon the reagents and the amount of hydrochloric acid required to neutralize the calcium chloride should be determined, as well as that required to acidify the quantity of the fertilizer sample taken for the determination. It will be necessary if the reagents are used in proportions other than the amounts suggested, to determine the effect of any such changes. In this work a blank of 0.35 cc. of 0.05N sodium hydroxide was obtained which, it will be noted, agrees with that found by Allen and Zies,

[1] *Z. physik. Chem.*, 1896, **20**: 547.

who have shown by very careful work that it is not due to boron in the glass of the apparatus.

SAMPLES.

The samples used in testing the method are those sent out by W. H. Ross to the collaborators this year, and the results obtained are as follows:

TABLE 1.

*Borax in mixed fertilizers.*

| SAMPLE NUMBER | ANHYDROUS BORAX | |
|---|---|---|
| | Found | Added |
| | *per cent* | *per cent* |
| 1 | 0.03 | 0.00 |
| 2 | 0.10 | 0.10 |
| 3 | 0.11 | 0.10 |
| 4 | 0.74 | 0.75 |

To further test this method, varying amounts of a solution containing a known amount of carefully recrystallized boric acid were added to 2 grams of the sample in the decomposition flask. The above Sample No. 1 was used and the results reported are corrected for the amount of anhydrous borax previously found.

TABLE 2.

*Recovery of known amounts of boric acid.*

| ANHYDROUS BORAX | |
|---|---|
| Added | Found |
| *per cent* | *per cent* |
| 0.05 | 0.05 |
| 0.10 | 0.10 |
| 0.15 | 0.14 |
| 0.20 | 0.20 |

No report was presented by G. F. Lipscomb, one of the associate referees on borax in fertilizers.

## THE DISTILLATION METHOD FOR THE ESTIMATION OF BORAX IN MIXED FERTILIZERS.

By J. M. BARTLETT (Agricultural Experiment Station, Orono, Me.).

As most members of this association know, the summer of 1919 was a notable one for both users and manufacturers of fertilizers on account

of the discovery that borax was present in some brands of mixed goods in sufficient quantity to be injurious to plants. Many complaints were received at the Maine Agricultural Experiment Station from farmers in Aroostook County that some fields of potatoes were not making normal growth and an investigation revealed the possibility that the brands of fertilizer on which they were planted carried considerable borax. Consequently, as many samples of the fertilizers used in the affected area as could be found were collected and sent to the Agricultural Experiment Station with the request that their borax content be determined as soon as possible. It was also considered desirable that the samples collected in the spring inspection be tested for borax. This increased the number of samples to about three hundred.

Since there is no official method for this determination, the writer was obliged to try out the various methods for the estimation of boron in other materials and test their applicability to mixed fertilizers. The Gladding and Thompson methods for the determination of borax in foods and food preservatives[1] seemed to promise well for the purpose and were tried first. In the Thompson method the material is first ignited with caustic soda to free it from organic matter, taken up with dilute hydrochloric acid and treated with calcium chloride, sodium hydroxide and limewater to remove phosphates, then filtered and the filtrate made acid to free the boric acid, which is titrated with 0.1N sodium hydroxide, using phenolphthalein as indicator after making the solution neutral to methyl orange and adding a neutral solution of glycerol. This method was quite simple to operate but, when tried on a fertilizer carrying a large amount of soluble phosphates and organic matter, it gave low results when known amounts of borax were added.

The Gladding method for the determination of borax in commercial preservatives, which is based on the fact that boric acid readily distils over with methyl alcohol vapor, was next tried. A very good grade of methyl alcohol was obtained which gave a blank of only 0.6 cc. of 0.1N sodium hydroxide on 10 cc. when distilled with the other reagents used, and when tried on several borax-free fertilizers, with known amounts of boric acid added, gave very satisfactory results. The boric acid was titrated in the alcohol distillate as directed in the Gladding method. Consequently, the whole operation required only 35 or 40 minutes and with two sets of stills one man could make fifteen to twenty determinations a day. The method was accurate to one or two tenths of a per cent and, as almost all fertilizers examined at that time, showing any borax by the qualitative test, contained from 0.3 to over 2 per cent it was sufficiently accurate for the writer's purpose and very rapid. Later on, however, the Bureau of Soils advised that a mixed fertilizer could not safely carry over 2 pounds (0.1 per cent) of anhydrous borax to the ton. Trials

[1] A. E. Leach. Food Inspection and Analysis. 4th ed., 1920, 884, 886.

made with fertilizers free from borax showed that some substances, acid to phenolphthalein, were volatilized with the alcohol which must be removed before the boric acid can be accurately titrated. It became necessary to modify the method so one could estimate hundredths instead of tenths of a per cent. The method now employed is given in detail below and, in the writer's laboratory, has proved to be accurate either with small or quite large amounts of borax. Late in the fall of 1919, the Bureau of Soils sent out a method developed in that laboratory which is somewhat similar to the Thompson method except that barium instead of calcium salts are used to separate phosphates from the boron. The method as applied to mixed fertilizers low in borax content is longer than the distillation method, and attempts have been made by some chemists, notably C. H. Jones of the Vermont Agricultural Experiment Station, to shorten it. The writer has not been able to recover as much of the borax by this method, or by other similar methods, as by the distillation method. Barium produces a heavier precipitate than calcium, inasmuch as it throws down all of the sulfuric acid present and would seem to offer a greater opportunity for loss of boron by occlusion.

After spending much time in trying out the various methods and modifications, both original and suggested, the writer prefers the Gladding distillation method for goods with high phosphoric acid and low boron content. It seems to be the most practical and accurate method when volatile organic acids both in the fertilizers and alcohol are eliminated. The method as conducted at the Maine Agricultural Experiment Station is as follows:

### APPARATUS.

The apparatus[1] consists of two 200 cc. round-bottomed flasks, a Liebig condenser, and a 200 cc. Erlenmeyer receiving flask. One of the 200 cc. round-bottomed flasks, No. 2, is supplied with a doubly perforated rubber stopper, through which passes a glass tube running to the bottom of the flask. The other hole is supplied with a short tube leading to the condenser. The other 200 cc. round-bottomed flask, No. 1, is fitted with a perforated rubber stopper and a short bent tube connected with a rubber tube leading to the long tube in Flask No. 2. The whole apparatus is supported by clamps and rings on two stands.

### DETERMINATION.

If the material to be examined is a mixed fertilizer or probably contains less than 2 per cent of anhydrous borax, weigh 5 grams into Flask No. 2. If the material is a chemical containing much more than 2 per cent of borax, use 2 grams. Then add 5 cc. of 50% phosphoric acid and 20 cc. of methyl alcohol and connect the flask with the condenser. Put 100 cc. of methyl alcohol in Flask No. 1, which is set in a water bath and connected with Flask No. 2. Put the receiving flask in place at the end of the condenser and apply sufficient heat to the water bath to keep a steady flow of bubbles of methyl alcohol passing through Flask No. 2. Some heat must also be applied to Flask No. 2 to keep the volume at about 25 cc. The lamps once regulated need

[1] A. E. Leach. Food Inspection and Analysis. 4th ed., 1920, 884.

very little attention and one person can easily run two distillations at once. Continue the distillation for about 30 minutes and distil 100 cc. When the distillation is complete, add 2–3 drops of phenolphthalein to the distillate and 5–10 cc. of 0.1N sodium hydroxide, or enough to give it a permanent pink color. Stopper the flask, shake well and connect at once with a regular alcohol still, supplied with a Hopkins' or similar bulb, distil off the alcohol and save for another determination. A water bath and not a lamp flame should be used for this purpose.

Leave the residue, which should be not less than 10 cc., in the flask, transfer to a platinum or porcelain dish, using as little water as possible, and evaporate to dryness on a steam or water bath. When dry, ignite below redness. Then acidify with a few drops of N hydrochloric acid, add 20–25 cc. of water and warm for 1–2 minutes on the steam bath; filter into a small flask, thoroughly wash and make up to about 50–75 cc., attach to an air-cooled condenser and gently boil for a few minutes to remove carbon dioxide. Add 3–4 drops of methyl red and 0.1N sodium hydroxide until the red color just disappears. Add about 1 gram of mannite, or less if but a small amount of boron is present. At this point if boric acid is present the solution will take on a pinkish color, the depth of color depending on the amount present, usually 0.01 or 0.02 per cent is sufficient to give the reaction if the solution has been carefully neutralized with the sodium hydroxide solution. Then add 2–3 drops of phenolphthalein and titrate the solution with standard 0.1N sodium hydroxide. A blank should be run with the reagents but if sodium hydroxide is free from carbon dioxide the blank should not be more than 0.2 cc. Recrystallized boric acid should be used to standardize the 0.1N sodium hydroxide.

The writer prefers the above method for the following reasons:

(1) The first distillation gives a more complete and clean separation of the boron from the other materials in the fertilizer than the writer has been able to obtain by any other method. There is no loss by occlusion or incomplete washing of heavy precipitates.

(2) The boron can be separated more quickly by distillation with methyl alcohol than by most of the precipitation methods and the actual time required by the chemist is less as the stills when once started need very little attention. With plenty of stills, many determinations can be made in a day.

(3) The same amount of substance can be taken, whether the amount of boron present is quite large or small.

(4) The blank from reagents is very small and not variable; if these are free from carbon dioxide, the blank should not be more than 0.02 per cent.

(5) Since only a small amount of sodium salts is present there is no spattering and therefore no chance for loss when the residue is ignited.

*Results obtained on fertilizers of known and unknown boron content.*

| FERTILIZER | BORAX ADDED | BORAX RECOVERED | |
|---|---|---|---|
| | | Distillation method | Ross-Deemer method |
| | *gram* | *gram* | *gram* |
| 5–10–0 | 0.005 | 0.0049 | 0.0031 |
| | 0.025 | 0.0236 | 0.0186 |
| | 0.050 | 0.0504 | 0.0390 |
| | 0.100 | 0.0992 | 0.0820 |
| 5–8–0 | 0.010 | 0.0101 | 0.0078 |
| | 0.005 | 0.0052 | 0.0040 |
| 4–8–0 | 0.100 | 0.1002 | 0.0895 |
| | 0.050 | 0.0485 | 0.0440 |
| 3–10–4 | 0.100 | 0.0960 | 0.075* |
| | 0.100 | 0 0960 | 0.076* |
| | 0.050 | 0.0460 | 0.034* |
| | 0.050 | 0.0480 | 0.035* |
| | 0.010 | 0.0092 | 0.012* |
| No. 1 | Unknown | 0.0022 | 0.0019 |
| No. 2 | Unknown | 0.0012 | 0.0009 |
| No. 3 | Unknown | 0.0008 | 0.0007 |
| No. 4 | Unknown | 0.0008 | 0.0006 |
| No. 5 | Unknown | 0.0005 | 0.0003 |
| No. 6 | Unknown | 0.0023 | 0.0018 |
| No. 7 | Unknown | 0.0021 | 0.0014 |

* Made by Jones' modification

# THE COMPOSITION AND PREPARATION OF A NEUTRAL SOLUTION OF AMMONIUM CITRATE[1].

By C. S. Robinson (Agricultural Experiment Station, E. Lansing, Mich.), *Associate Referee on the Preparation of Ammonium Citrate.*

The following report is submitted in accordance with the recommendation approved by the association at its 1919 meeting[2] "that the referee be instructed to determine the exact composition of a strictly neutral solution of ammonium citrate and report at the next meeting of the association with recommendation as to the preparation of such a solution".

Prior to his notification of the above action by the association the associate referee had taken up the investigation of this subject and had prepared the results for publication[3]. Further details of the work discussed in the present report may be obtained from that publication.

## THE COMPOSITION OF A STRICTLY NEUTRAL SOLUTION OF AMMONIUM CITRATE.

From the very first proposal of the use of this reagent, there has existed a confusion of the term "neutral", as applied to the solution itself and

[1] Presented by R. N. Brackett.
[2] *J. Assoc. Official Agr. Chemists*, 1921, 4: 565.
[3] Mich. Agr. Expt. Sta. Tech. Bull. 46: (1919).

to the salt. This was due to a failure to recognize the fact that, owing to dissociation and hydrolysis, a solution of the "neutral", or better the normal, salt of ammonia and citric acid is not neutral but alkaline, *i. e.*, the concentration of the hydroxyl ions exceeds that of the hydrogen ions.

Attempts have been made to fix the composition of a truly neutral solution but without much success and as Hand has pointed out, "any analytical method in a large measure leaves the question in its former condition, because we must first prepare the neutral solution before we can ascertain the precise amounts of citric acid and of ammonia that will reproduce it"[1].

McCandless[2], acting in accordance with instructions of the association, attempted to determine the relation between the reaction and composition of several solutions sent to him by testing them for neutrality to corallin and also determining the ratios of ammonia to citric acid in them. Of nine solutions submitted, three proved to be neutral to the indicator used. The ratios in these were respectively 1: 3.803, 1: 3.816 and 1: 3.808, giving an average of 1 to 3.809.

Of the solutions analyzed by McCandless, the three which were neutral to corallin were actually very close to the point of absolute neutrality, much closer, in fact, than the one which he selected as a standard. Here again, however, is a case of the confusion of the two solutions, one neutral itself in reaction and the other a solution of the neutral salt.

The development of the methods for determining the concentration of hydrogen ions in solutions gave the first method for accurately preparing a truly neutral solution, the determination of whose composition would serve for its reproduction. Using the colorimetric method outlined below, but with some variations in technique, a number of such neutral solutions was prepared and analyzed. The procedure used in their preparation was as follows:

One hundred and ten gram portions of citric acid were weighed into 700 cc. flasks, each dissolved in 75 cc. of water and mixed with concentrated ammonia to bring the reaction to a pH of 6.6–6.8. For final neutralization, each solution was transferred to a 500 cc. graduate, diluted to a density of approximately 1.10 and mixed with equal quantities of phenol red indicator. The same volume of standard buffer solution with a pH of 7.0 was placed in a similar graduate and mixed with the same amount of indicator. To the citrate solutions 2 N ammonium hydroxide was then added until the colors checked that of the standard solution whose volume had been equalized with distilled water. The reaction was finally checked by removing a few cc. of the citrate solution, diluting and checking against some of the standard in the comparator. The neutralized solutions were then returned to the flasks and

[1] U. S. Bur. Chem. Bull. 132: (1910), 9.
[2] *Ibid.*, 122: (1908), 147.

their densities adjusted to 1.0900 ± 0.0001 at 20° C. ± 0.5 determined with a pycnometer.

For analysis, 25 cc. samples were diluted to 250 cc. and 10 cc. portions were used. The ammonia was estimated by the distillation method and the citric acid by titration after the addition of formaldehyde, all determinations being made in triplicate. The averages are given in the following table:

TABLE 1.

*Analysis of ammonium citrate solutions.*

| SOLUTION | AMMONIA PER LITER | ANHYDROUS CITRIC ACID PER LITER | RATIO OF AMMONIA TO ANHYDROUS CITRIC ACID |
|---|---|---|---|
| | *grams* | *grams* | |
| 1 | 45.39 | 171.83 | 1 : 3.785 |
| 2 | 45.36 | 172.66 | 1 : 3.807 |
| 3 | 45.37 | 172.02 | 1 : 3.791 |
| 4 | 45.35 | 171.96 | 1 : 3.791 |
| 5 | 44.90 | 171.06 | 1 : 3.809 |
| 6 | 45.39 | 171.70 | 1 : 3.783 |
| 7 | 45.68 | 173.05 | 1 : 3.788 |
| 8* | 45.20 | 171.70 | 1 : 3.798 |
| Average..... | 45.33 | 172.00 | 1 : 3.794 |

* Neutralized by ordinary technique.

While such differences may appear to be large, it should be stated that the extreme readings for the whole series were 26.41 and 26.87 cc. of 0.1N hydrochloric acid for the ammonia determinations and 26.72 and 27.03 cc. of 0.1N sodium hydroxide for the citric acid estimations. *A "neutral" solution of ammonium citrate may then be defined as one in which the ratio of ammonia to anhydrous citric acid is 1 to 3.794.* At 20° C. such a solution containing 45.33 grams of ammonia and 172.00 grams of anhydrous citric acid per liter will have a density of 1.09.

## THE PREPARATION OF A STRICTLY NEUTRAL SOLUTION OF AMMONIUM CITRATE.

In some biochemical work involving the determination of hydrogen ion concentrations, the writer had occasion to make use of the methods devised by Clark and Lubs[1] for the preparation of bacterial culture media. It occurred to him that these methods could be applied equally well to the control of ammonium citrate solutions as had already been done in principle by Eastman and Hildebrand[2]. The only differences between the method of the latter investigators and the one to be described are

[1] *J. Bact.*, 1917, 2: 1.
[2] *J Ind. Eng. Chem.*, 1914, 6: 577.

in the indicator and the standard solutions used, changes which, however, seem to greatly increase the accuracy and ease of manipulation of the process.

Both procedures are based upon the method for determining colorimetrically the hydrogen ion concentration of a solution, *i. e.*, the reactions of solutions. In brief, this consists in preparing a series of solutions, whose compositions fix their reactions which are originally determined electrometrically, adding to definite quantities of these solutions equal quantities of a suitable indicator and comparing with them the color produced by an equal concentration of the same indicator in the solution to be tested. The basis of the method is found in the fact that indicators change color not abruptly but through a definite range of hydrogen ion concentration and that the range of reaction through which the change of color is observable differs for different indicators. Thus, by the proper choice of indicators, any region of reaction from normal hydrogen ion concentration to normal hydroxyl ion concentration may be studied.

The choice of indicator is governed by its availability, its effective range and the vividness of its color change; the selection of the standard buffer solutions by the ease with which they can be accurately duplicated and their range of reaction.

For the preparation of a neutral solution, an indicator must be chosen whose maximum color change occurs at approximately the neutral point. In the recently developed sulfonphthalin series this point is included in the range of phenolsulfonphthalin or phenol red. Hence this indicator was tested as a substitute for the less brilliant corralin and cochineal.

It is proposed to use as a standard buffer solution one prepared from potassium dihydrogen phosphate and sodium hydroxide according to Clark and Lubs. The complete series covers a range from pH 5.8 to pH 8.0 in steps of 0.2. The following table shows the compositions of the various mixtures, together with their reactions in terms of pH[1].

TABLE 2.

*$KH_2PO_4$—NaOH Mixtures.*

| | | | |
|---|---|---|---|
| 5.8 | 50 cc. M/5 $KH_2PO_4$ | 3.72 cc. M/5 NaOH | Dilute to 200 cc. |
| 6.0 | 50 cc. M/5 $KH_2PO_4$ | 5.70 cc. M/5 NaOH | Dilute to 200 cc. |
| 6.2 | 50 cc. M/5 $KH_2PO_4$ | 8.60 cc. M/5 NaOH | Dilute to 200 cc. |
| 6.4 | 50 cc. M/5 $KH_2PO_4$ | 12.60 cc. M/5 NaOH | Dilute to 200 cc. |
| 6.6 | 50 cc. M/5 $KH_2PO_4$ | 17.80 cc. M/5 NaOH | Dilute to 200 cc. |
| 6.8 | 50 cc. M/5 $KH_2PO_4$ | 23.65 cc. M/5 NaOH | Dilute to 200 cc. |
| 7.0 | 50 cc. M/5 $KH_2PO_4$ | 29.63 cc. M/5 NaOH | Dilute to 200 cc. |
| 7.2 | 50 cc. M/5 $KH_2PO_4$ | 35.00 cc. M/5 NaOH | Dilute to 200 cc. |
| 7.4 | 50 cc. M/5 $KH_2PO_4$ | 39.50 cc. M/5 NaOH | Dilute to 200 cc. |
| 7.6 | 50 cc. M/5 $KH_2PO_4$ | 42.80 cc. M/5 NaOH | Dilute to 200 cc. |
| 7.8 | 50 cc. M/5 $KH_2PO_4$ | 45.20 cc. M/5 NaOH | Dilute to 200 cc. |
| 8.0 | 50 cc. M/5 $KH_2PO_4$ | 46.80 cc. M/5 NaOH | Dilute to 200 cc. |

[1] *J. Bact.*, 1917, 2: 26

Unless some means of checking the first solutions made from it are accessible, the potassium dihydrogen phosphate should be recrystallized four or five times. This product may then be kept as a special reagent for future use and the solutions made from it by carefully weighing out the desired quantities.

The sodium hydroxide solution is prepared from carbonate-free material and standardized in the usual manner. It should be kept in a paraffined bottle and protected from the carbon dioxide of the air.

From these stock reagents solutions may be prepared repeatedly having reactions so constant that no difference in color can be detected between lots made at different times. Thus, once the reliability of a given stock is established, the accuracy of the results with it may be regarded as fixed.

For the purpose in hand the whole series is of course unnecessary. It is well, however, to prepare two extra solutions, one on either side of the one to which the unknown solution is to be compared. This permits of a check on the accuracy of the standard, whose color should be intermediate between the colors of the other two. It also allows a more accurate and rapid adjustment of the reaction of the unknown, the color of which may be made roughly to match that of one of the solutions above or below the one finally sought and then carefully brought to the ultimately desired point. If, on the other hand, the end point is slightly overreached in the process it may be detected more easily and the magnitude of the error approximately judged by comparison with the third solution.

In this laboratory comparisons are made in test tubes 7 × $\frac{7}{8}$ inches placed in a comparator similar to that described by Dernby and Avery[1].

The following procedure is recommended for the preparation of neutral ammonium citrate solutions. When carefully carried out it yields solutions having densities of 1.09 in which the ratio of ammonia to anhydrous citric acid is 1 to 3.794 ± 0.015.

*Neutral ammonium citrate solution.*—For every liter of solution required dissolve 172.00 grams of anhydrous or 188.13 grams of crystallized citric acid in approximately 700 cc. of water; nearly neutralize with commercial ammonium hydroxide; cool; measure the volume of the solution or make it up to a convenient volume, taking care to keep the density above 1.09; make exactly neutral, testing as follows: With a pipet transfer 5 cc. of the citrate solution to a 7 × $\frac{7}{8}$ inch test tube and dilute to 20 cc. with distilled water. Add from a dropping bottle 5 drops of a 0.02% solution of phenol red (prepared by diluting a 0.4% stock solution containing 0.1 gram of the dye ground in an agate mortar with 5.7 cc. of 0.05N sodium hydroxide and made up to 25 cc.). From a buret run in standard ammonia solution until the color approximates that of an equal volume of a neutral standard phosphate solution (prepared by mixing 50 cc. M/5 dihydrogen potassium sulfate and 29.63 cc. M/5 sodium hydroxide and making up to 200 cc.) contained in a similar test tube and with the same concentration of

[1] *J. Exp. Med.*, 1918, 28: 348.

indicator. Complete the process by carefully adding the standard ammonia solution in small amounts and comparing the colors in the colorimeter. From the amount of ammonia solution required to produce in the sample a color which exactly matches that of the standard, calculate the amount required to neutralize the rest of the solution.

Add this calculated amount of ammonia to the original solution and check its reaction against that of the neutral standard, using the technique described above. If the colors match, dilute the solution to 1 liter for every 172.00 grams of anhydrous citric acid used.

Finally check the composition of the solution by determining the ratio of ammonia to citric acid by the method of Patten and Marti[1] and its density by means of a hydrometer.

RECOMMENDATIONS.

It is recommended—

(1) That a neutral solution of ammonium citrate be considered as one in which the ratio of ammonia to anhydrous citric acid is 1 to 3.794 and which shall contain 45.33 grams of ammonia and 172.00 grams of anhydrous citric acid per liter at 20° C.

(2) That the method described on page 98, be made the official method for the preparation of neutral ammonium citrate.

H. P. Nelligan (American Glue Company, Boston, Mass.) presented a paper on "Limitations of the Present Official Methods of Analysis for Insoluble Phosphoric Acid in Dicalcium Phosphate". Considerable discussion followed the reading of this paper with the result that it was re-referred to Committee A and H. D. Haskins invited to sit with the committee when it met. Committee A, however, failed to report upon the matter.

## REPORT ON AVAILABLE PHOSPHORIC ACID IN PRECIPITATED PHOSPHATES.

By H. D. Haskins (Agricultural Experiment Station, Amherst, Mass.), *Associate Referee.*

At the request of R. N. Brackett, General Referee on Fertilizers, the writer consented to act in the capacity of Associate Referee on Available Phosphoric Acid in Precipitated Phosphates. The studies which have been made on this subject during the year have been carried on in the laboratories of the Massachusetts Agricultural Experiment Station and no attempt has been made to secure results from other experiment stations or fertilizer control chemists for the reason that very little precipitated phosphate seems to be distributed to the farmer when unmixed

[1] *J. Ind. Eng. Chem.*, 1913, 5: 567.

with other materials except in the Connecticut River tobacco districts and among the citrus fruit growers of Florida and possibly some other Southern States. Your associate referee did not feel justified in asking for the cooperation of chemists who were already overworked with other problems and who would have only a passing interest in this particular subject. Some data, however, have been secured from H. P. Nelligan, a representative of the American Glue Company, which will form a part of this report. It may be of interest to add that the Belgian and German chemists use what is known as the Petermann method, employing 1 gram of the precipitated phosphate with 100 cc. of alkaline citrate of ammonia. Italian and French chemists also have special methods for testing precipitated phosphate. The Italian method employs 100 cc. of neutral citrate of ammonia with a ½-gram charge, while the French method calls for 40 cc. of a slightly alkaline solution of citrate of ammonia with a ½-gram charge. The material which has served for the studies which make up this report was supplied by the American Glue Company of Boston and represented their ordinary run of precipitated phosphate. It tested 39.22 per cent of total phosphoric acid (average of seven determinations).

## SCOPE OF THE EXPERIMENT.

The experiment was planned to show: (1) A comparison of results obtained by the use of a 1- and 2-gram charge with a manipulation as outlined in the official methods for the determination of soluble and insoluble phosphoric acid in fertilizers[1]; (2) a comparison of results obtained by the use of 100 cc., 150 cc. and 200 cc. of neutral citrate of ammonia on a 2-gram charge; (3) the effect of two successive treatments, each employing 100 cc. of neutral citrate of ammonia, on a 2-gram charge of the precipitated phosphate (the properly washed residue obtained after treating 2 grams of the phosphate was introduced into an Erlenmeyer flask together with the filter paper and the whole treated with a second application of 100 cc. of neutral citrate of ammonia); (4) a study of the behavior of the phosphate when mixed with other crude stock materials, as in the manufacture of a complete fertilizer (a 4-8-4 mixture was made up by the use of nitrate of soda, dried blood, sulfate of potash and magnesia, precipitated bone, and peat filler; results were obtained by treating a 1- and 2-gram charge with neutral citrate of ammonia according to the association methods); (5) an experiment was run to note the effect of maintaining a constant temperature at 65° C. of the neutral citrate solution while filtering and washing after the treatment of 2 grams of the phosphate with 100 cc. of neutral citrate of ammonia. It was thought that perhaps the high insoluble phosphoric acid tests that were obtained when 2 grams of the phosphate were treated with 100 cc. of neutral citrate of ammonia solution were due to a partial

[1] *Assoc. Official Agr. Chemists, Methods.* 2nd ed., 1920, 4-5.

TABLE 1.

*Results obtained in a study of methods for the determination of available phosphoric acid in precipitated phosphate.*

| ANALYST | Total phosphoric acid | Water-soluble phosphoric acid* 2-gram sample | CITRATE-SOLUBLE PHOSPHORIC ACID | | CITRATE-INSOLUBLE PHOSPHORIC ACID BY OFFICIAL METHOD | | CITRATE-INSOLUBLE PHOSPHORIC ACID 2-GRAM SAMPLE | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | 2-gram sample | 1-gram sample | 2-gram sample | 1-gram sample | 150 cc. of neutral citrate of ammonia | 200 cc. of neutral citrate of ammonia | Successive treatments with 100 cc. each of citrate of ammonia |
| | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | |
| L. S. Walker, Amherst, Mass | 39.22 | 2.37† | 28.94† | 34.45† | 7.91† | 2.40† | 5.33† | 2.88† | ..... |
| A. M. Clarke, Amherst, Mass | 39.22 | 2.39‡ | 29.39‡ | 35.16‡ | 7.44‡ | 1.67‡ | .... | .... | trace |

* Average of 3 tests using 1-gram sample, 2 60 per cent.
† Average of 3 tests.
‡ Average of 4 tests

precipitation of the dicalcic phosphate from the highly saturated solution of neutral citrate of ammonia during the filtration process.

Results have been obtained on these studies by two analysts, Walker and Clarke. The tabulated results are given in Table 1.

NOTE 1.—In the 4-8-4 fertilizer mixture where the precipitated phosphate was used as the only source of phosphoric acid, when employing 100 cc. of neutral citrate of ammonia, a 2-gram charge gave citrate-insoluble phosphoric acid, 0.08 per cent, and a 1-gram charge gave 0.10 per cent.

NOTE 2.—In the filtration of all of the citrate solutions double filters were used; quality C. S. & S. No. 597, 11 cm. Gentle suction was employed with a platinum cone.

NOTE 3.—The material showed the presence of 40.62 per cent of calcium oxide and 7.24 per cent of water.

The following determinations were made by the official method[1], using Whatman paper No. 2, gentle suction with a platinum cone.

TABLE 2.

*Results obtained in the determination of citrate-insoluble phosphoric acid.*

(Analyst, H. P. Nelligan.)

| SAMPLE | CITRATE-INSOLUBLE PHOSPHORIC ACID | |
|---|---|---|
| | 2-gram charge | 1-gram charge |
| | *per cent* | *per cent* |
| A | 4.31 | 2.10 |
| B | 5.02 | 2.80 |
| C | 3.40 | 1.90 |
| D | 4.10 | 2.03 |
| E | 3.80 | 1.90 |
| F | 3.04 | 1.20 |

[1] *Assoc. Official Agr. Chemists, Methods.* 2nd ed., 1920, 4.

## DISCUSSION OF RESULTS.

The results obtained by both analysts at the Massachusetts Agricultural Experiment Station show a considerably higher availability of the phosphoric acid with a 1-gram charge than when 2 grams are used, as called for in the official methods. The same is true on the work of the analyst from the American Glue Company when working on six different samples of precipitated phosphate. All of these results bear out the observations of the writer on earlier studies of this class of materials. The additional results obtained by Walker in the use of 150 and 200 cc. of citrate of ammonia with a 2-gram charge of phosphate also show an increasing amount of available phosphoric acid, the use of 200 cc. of neutral citrate of ammonia with a 2-gram charge of phosphate giving about the same availability as 100 cc. when using a 1-gram charge. The results obtained by Clarke, using a 2-gram charge with two successive treatments of 100 cc. each of neutral citrate of ammonia, show 100 per cent availability of the phosphoric acid. The results obtained by Walker on the 4-8-4 fertilizer mixture which contained all of its phosphoric acid in the form of precipitated phosphate would indicate that when this product was used as a source of phosphoric acid in the average mixed fertilizer its availability by the official method would be very high (over 99 per cent).

The experiment which was run to study the effect of maintaining a constant temperature of 65° C. of the citrate solution during filtration resulted in having but little effect on the availability of the phosphoric acid in the product. The results at hand would, therefore, indicate that the low availability of the phosphoric acid in precipitated phosphate when run by the official method, using a 2-gram charge with 100 cc. of neutral citrate of ammonia, was due to a saturated citrate solution, resulting from the large amount of dicalcic phosphate present.

With regard to the availability of the phosphoric acid in precipitated phosphate as measured by vegetation tests, it may be said that a 2-year test on the phosphate fields at the Massachusetts Agricultural Experiment Station with corn and grass and clover shows a satisfactory crop, as may be seen from the following results:

TABLE 3.

*Results of a 2-year test on phosphate fields.*

| | PHOSPHATE | 1919 CROP CORN YIELD PER ACRE | | 1920 GRASS AND CLOVER PER ACRE |
|---|---|---|---|---|
| | | GRAIN | STOVER | |
| | | *bushels* | *pounds* | *pounds* |
| Plot 1 | No phosphoric acid | 83.0 | 4840 | 6902 |
| Plot 2 | Tennessee rock | 58.1 | 4560 | 6632 |
| Plot 3 | Acid phosphate | 51.8 | 6160 | 8122 |
| Plot 4 | Florida soft rock | 55.0 | 4680 | 7868 |
| Plot 5 | Basic slag | 72.9 | 8280 | 7738 |
| Plot 6 | Steamed bone | 78.8 | 3840 | 8594 |
| Plot 7 | No phosphoric acid | 55.5 | 3640 | 7896 |
| Plot 8 | Precipitated bone* | 78.9 | 7440 | 8208 |
| Plot 9 | Raw bone | 77.4 | 8520 | 7438 |
| Plot 10 | Tennessee rock | 64.8 | 7120 | 8136 |
| Plot 11 | Steamed bone | 77.7 | 6920 | 8452 |
| Plot 12 | Acid phosphate | 72.4 | 5960 | 7836 |
| Plot 13 | No phosphoric acid | 54.0 | 3480 | 6382 |

* Previous to 1919 this plot had received dissolved bone black as phosphoric acid source.

It may be added that in general farm practice among the tobacco growers of the Connecticut Valley the precipitated phosphate has for years received the preference, even with an advance in price, over acid phosphate.

## RECOMMENDATION.

Your associate referee is convinced that the present official method for the determination of available phosphoric acid does not give full justice to this class of materials, and he would therefore recommend that the determination of insoluble phosphoric acid in precipitated phosphate be carried out according to the present official method for the determination of insoluble phosphoric acid in fertilizers[1] with the exception that a 1-gram charge be employed and that a quality of filter paper corresponding to C. S. & S. No. 597 be used, together with a perforated platinum cone and gentle suction, in the filtration of the citrate solution after treatment.

[1] *Assoc. Official Agr. Chemists, Methods.* 2nd ed., 1920, 4.

# A MODIFIED METHOD FOR THE DETERMINATION OF PHOSPHORIC ACID.

By A. W. CLARK and R. F. KEELER (Agricultural Experiment Station, Geneva, N. Y.).

During the past four years considerable work has been done in the writers' laboratory on a gravimetric method for the determination of phosphoric acid, based on the weight of the ammonium phosphomolybdate precipitate. While it is not claimed that there is any new principle involved, the main differences between the method employed by the writers and the usual procedure are: the precipitation at room temperature; drying the precipitate for 2 hours at 120° C.; and the factor for phosphoric acid.

The investigation of the first two years was of a preliminary nature. A slightly different factor was used from that finally adopted and drying of the precipitate was effected at a temperature of 110° C., maintained for a period of 1 hour. Some 2500 analyses were made and compared with the official magnesium gravimetric method, from which a factor was derived.

Upon drying the yellow precipitate for 2 hours at 120° C., the factor variation was greatly reduced and gave better agreement between duplicate determinations. The factor was found to vary slightly from the one previously used. The method employed is as follows:

Dissolve 2 grams of the sample in 30 cc. of concentrated nitric acid and 10 cc. of hydrochloric acid. Boil until solution is complete, cool, dilute to a volume of 200 cc., mix and pour through a dry filter. Neutralize a portion equivalent to 0.25 gram with ammonium hydroxide and acidify with nitric acid. Add 50 cc. of 20% ammonium nitrate solution; and then sufficient ammonium molybdate solution to completely precipitate the phosphorus (35 cc. added for samples containing between 6 and 12 per cent of total phosphoric acid). Do not heat the solution but allow it to stand overnight at room temperature. Filter on a weighed porcelain Gooch crucible of 25 cc. capacity dried at 120°C. Wash eight times with 2% nitric acid, filling the crucible about half full each time. Wash twice with cold distilled water, dry for 2 hours at 120°C., and weigh.

Determinations in duplicate by one of the writers, using the official method, and single determinations by the other, using the above method, were made upon 95 samples.

59 samples, or 62.1 per cent, showed a difference of 0.10 per cent or less.
27 samples, or 28.4 per cent, showed a difference between 0.10 and 0.20 per cent.
9 samples, or 9.5 per cent, showed a difference of 0.20 per cent or more.
No samples showed a difference of 0.31 per cent or more.

The factor used for converting the weight of the ammonium phosphomolybdate to phosphoric acid is 0.03723.

For the determination of insoluble phosphoric acid, an aliquot of 50 cc., corresponding to 0.50 gram, is recommended. In this case 25 cc. of a 40 per cent solution of ammonium nitrate are added after neutralizing with ammonium hydroxide and acidifying with nitric acid.

## REPORT ON NITROGEN.

By I. K. PHELPS (Bureau of Chemistry, Washington, D. C.), *Associate Referee on Nitrogen in Fertilizers.*

It was recommended in 1919[1] that a study of the du Pont nitrometer be made in order to determine its practicability for the estimation of nitric and nitrous nitrogen. An attempt was made to secure collaborators to carry out this study, but the war prevented securing the desired number. A number of those who signified their willingness to do this work failed to report their results. The few reports received are not presented since they are insufficient to be convincing.

The writer had analyses made, among other samples, of a check sample of potassium nitrate of the E. I. du Pont de Nemours & Company, duplicates of which were used to check up the various nitrometers in use at the du Pont Laboratories. The weights of nitrous oxide found on four estimations on a 1-gram sample were 0.29667 gram; 0.29710 gram; 0.29732 gram; 0.29732 gram. The average of the four samples was 0.29710 gram of nitric oxide gas which, calculated to potassium nitrate, is 100.07 per cent of that taken.

Beckett[2] has investigated the du Pont nitrometer as applied to the analysis of explosives. He concludes that there is an appreciable quantity of nitrogen and nitrous oxide gas left in the sulfuric acid residue after the estimation of nitrogen in du Pont's high nitrogen gun cotton, gun cotton, highly soluble nitrocellulose, blasting soluble nitrocellulose and sodium nitrate.

In conclusion, Beckett states that the nitrometric estimation of nitrogen in nitrocellulose is invariably too low. He has found that the time which is allowed to elapse between the introduction of the nitrocellulose and sulfuric acid into the nitrometer and the shaking has a great influence on the results, in the case of nitrocellulose. This effect is less in the case of inorganic compounds. The truest results are obtained by using 15 cc. of 92.5 to 94 per cent sulfuric acid and by allowing 15 minutes to elapse between the introduction of the nitrocellulose and acid into the nitrometer and shaking. In this case he states that the error is approximately 0.7 per cent.

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[1] *J. Assoc. Official Agr. Chemists*, 1921, 4: 365.
[2] *J. Chem. Soc.*, 1920, 117: 220.

## RECOMMENDATIONS.

Since the du Pont nitrometer can be used only in the analysis of inorganic nitrates and mixed acids and then only by approximating the true results through the approximate elimination of the errors in the method by standardization with a pure nitrate, and since a better method for fertilizer control appears available, it is recommended—

(1) That the study of the du Pont nitrometer be abandoned.

(2) That the referee for 1921 be directed to study the Devarda alloy method[1] as applied to the determination of nitric and nitrous acids in fertilizers.

TABLE 1.

*Analysis of check sample of potassium nitrate from E. I. du Pont de Nemours & Company.*

(Analyst, L. J. Jenkins.)

| NITROGEN FOUND | EQUIVALENT AMMONIA | EQUIVALENT POTASSIUM NITRATE |
|---|---|---|
| *per cent* | *per cent* | *per cent* |
| 13.85 | 16.84 | 99.93 |
| 13.87 | 16.86 | 100.07 |
| 13.88 | 16.88 | 100.14 |
| 13.88 | 16.88 | 100.14 |

TABLE 2.

*Sample of potassium nitrate recrystallized in the Bureau of Chemistry.*

| NITROGEN FOUND | EQUIVALENT AMMONIA | EQUIVALENT POTASSIUM NITRATE |
|---|---|---|
| *per cent* | *per cent* | *per cent* |
| 13.81 | 16.79 | 99.64 |
| 13.86 | 16.85 | 100.00 |

# BORIC ACID FOR NEUTRALIZING AMMONIA IN NITROGEN DETERMINATIONS[2].

By H. D. SPEARS (Agricultural Experiment Station, Lexington, Ky.).

Scales and Harrison[3] give results that suggest that boric acid for neutralizing the ammonia in nitrogen determinations[4] would be desirable for feeding stuffs analysis. The method according to Scales and Harrison has the following advantages:

[1] *Chem. Ztg.*, 1892, 16: 1952; *J. Ind. Eng. Chem.*, 1919, 11: 306; 1920, 12: 352.
[2] Presented by I. K. Phelps.
[3] *J. Ind. Eng. Chem.*, 1920, 12: 350.
[4] *Z. angew. Chem.*, 1913, 26: 231.

1.—It does away with the occasional errors that arise from slight mistakes in measuring the sulfuric acid into the receiving flask.

2.—As the boric acid solution need be measured only approximately, much time can be saved, and an unskilled helper can measure it into the receiving flasks.

3.—By proper adjustment of the strength of the standard acid and the weights of the samples taken the percentage of nitrogen can be read directly from the buret.

4.—It is necessary to prepare accurately only one standard solution, *i. e.*, the sulfuric acid for titrating.

Ninety-five mg. of nitrogen as ammonia can be recovered in the distillate when 50 cc. of 4% boric acid is used.

Bromophenol blue is a better indicator than those used by the other investigators.

In laboratories where it is necessary to run a great number of nitrogen determinations, the above-mentioned advantages are highly desirable, though Item No. 3 is not peculiar to the boric acid method.

In order to have a fair comparison of the two methods, sixty laboratory samples of feeding stuffs, taken in the order in which they were received, were run by both methods, a modified Kjeldahl method using sulfuric acid as the neutralizing agent (the regular laboratory method), and the boric acid modification of the Kjeldahl method.

### LABORATORY METHOD.

Digest 0.7005 gram of the ground sample, 10 grams of powdered sodium sulfate, 1 cc. of saturated copper sulfate solution and 30 cc. of concentrated sulfuric acid for 3 hours. Cool, dilute, add about 90 cc. of a mixture of saturated caustic soda and potassium sulfide (40 grams of potassium sulfide per liter) and then a small amount of 20-mesh zinc, and distil into sulfuric acid. Titrate the excess sulfuric acid with 0.1N sodium hydroxide, using 4 drops of a 1 per cent solution of alizarin as the indicator. Measure the sulfuric acid with an automatic pipet. A blank is run, using all reagents. It is not necessary to know the normality of the sulfuric acid used. The difference between the cc. reading of the blank and the determination gives the amount of 0.1N sodium hydroxide equivalent to the nitrogen. Instead of using a 0.1N sodium hydroxide solution, a solution, 1 cc. of which is equivalent to 1 mg. of nitrogen, may be used, and the weight of the sample taken may be 1 gram instead of 0.7005 gram so that the per cent of nitrogen can be read directly from the buret.

### BORIC ACID MODIFICATION.

Conduct the digestion as above, receive the distillate in 50 cc. of a 4 per cent solution of boric acid. Titrate as suggested by Scales and Harrison (artificial light) and use 6 drops of a 0.04 solution of bromophenol blue as the indicator. A blank, using all reagents, is run and subtracted from the determination. Tenth-normal sulfuric acid was used to titrate the distillate.

TABLE 1.

*Nitrogen in feeding stuffs.*

| DESCRIPTION OF SAMPLE | BORIC ACID NEUTRALIZING AGENT | SULFURIC ACID NEUTRALIZING AGENT |
|---|---|---|
| | *per cent* | *per cent* |
| Hog feed | 2.95 | 2.92 |
| Mixed wheat feed | 2.80 | 2.78 |
| Hog feed | 3.37 | 3.40 |
| Shipstuff | 2.66 | 2.68 |
| Mixed wheat feed | 2.67 | 2.68 |
| Wheat middlings | 2.59 | 2.56 |
| Barley mixed feed | 2.32 | 2.36 |
| Dry mash | 2.59 | 2.60 |
| Wheat shorts | 3.03 | 3.00 |
| Wheat middlings | 2.63 | 2.64 |
| Wheat mixed feed | 2.85 | 2.88 |
| Cracked corn | 1.43 | 1.48 |
| Dairy feed | 2.53 | 2.58 |
| Mixed middlings | 2.57 | 2.58 |
| Cottonseed meal | 6.40 | 6.32 |
| Cottonseed meal | 6.67 | 6.62 |
| Cottonseed meal | 6.33 | 6.26 |
| Mixed feed | 2.54 | 2.56 |
| Mixed feed | 2.62 | 2.60 |
| Horse feed | 1.68 | 1.68 |
| Ground oats | 2.00 | 2.04 |
| Hog ration | 2.79 | 2.92 |
| Dairy feed | 2.71 | 2.72 |
| Dairy feed | 2.24 | 2.22 |
| Dairy feed* | 3.83 | 3.92 |
| Cracked corn* | 1.43 | 1.42 |
| Velvet bean feed* | 2.81 | 2.76 |
| Horse feed* | 1.73 | 1.76 |
| Dairy feed* | 3.33 | 3.30 |
| Winter wheat middlings* | 2.29 | 2.30 |
| Mixed feed | 2.50 | 2.50 |
| Horse and mule feed | 1.55 | 1.60 |
| Mixed feed | 2.62 | 2.60 |
| Mill feed | 2.37 | 2.44 |
| Mixed feed | 2.38 | 2.38 |
| Sewage sludge | 2.39 | 2.40 |
| Horse and mule feed | 2.05 | 2.02 |
| Hog and dairy feed | 2.79 | 2.78 |
| Hen feed | 1.32 | 1.34 |
| Horse and mule feed | 1.57 | 1.56 |
| Mixed feed | 2.17 | 2.18 |
| Alfalfa and cow feed | 1.63 | 1.60 |
| Mixed feed | 2.56 | 2.56 |
| Hog feed | 1.89 | 1.94 |
| White hominy feed | 1.83 | 1.84 |
| Horse and mule feed | 1.55 | 1.48 |
| Alfalfa and molasses | 2.08 | 2.08 |
| Cottonseed meal | 6.65 | 6.54 |
| Cottonseed feed | 3.09 | 3.02 |
| Horse and mule feed | 1.97 | 1.96 |
| Wheat bran | 2.59 | 2.56 |
| Horse and mule feed | 1.70 | 1.74 |
| Dairy feed | 3.12 | 3.18 |
| Horse feed | 1.71 | 1.72 |
| Hen feed | 1.47 | 1.48 |
| Little chick feed | 1.97 | 2.00 |
| Dairy feed | 2.97 | 3.00 |
| Digester tankage | 9.15 | 9.16 |
| Pig meal | 2.69 | 2.72 |
| Hog feed | 2.19 | 2.20 |
| Average | 2.75 | 2.75 |

* See Table 2.

Bromocresol purple, an indicator giving two very decided colors—purple to yellow—from alkalinity to acidity—was also tried in the same manner as bromophenol blue with the following good results:

TABLE 2.

*Nitrogen in feeding stuffs, bromocresol purple as an indicator.*

| DESCRIPTION OF SAMPLE | BORIC ACID FIXING AGENT |
|---|---|
| | *per cent* |
| Dairy feed | 3.86 |
| Cracked corn | 1.46 |
| Velvet bean feed | 2.84 |
| Horse feed | 1.75 |
| Dairy feed | 3.36 |
| Winter wheat middlings | 2.32 |

From the figures in the tables it is seen that the methods are quite comparable. The writer is of the opinion that there is not much choice between the two methods. It is true that errors might occur in measuring the sulfuric acid used as the neutralizing agent, but titrating with artificial light, although a satisfactory end point is obtained, is hardly as desirable. In one case, an accurately standardized acid is used, and in the other, an accurately standardized alkali.

## THE KJELDAHL NITROGEN METHOD AND ITS MODIFICATIONS[1].

By A. E. PAUL[2] and E. H. BERRY (U. S. Food and Drug Inspection Station, Transportation Building, Chicago, Ill.).

Because of the importance of nitrogen in all matters pertaining to nutrition, the determination of this element is one of the most important if not the most important, to every food analyst. In the case of feeding stuffs particularly, the percentage of nitrogen frequently forms the basis for the evaluation of the material. Unfortunately, the methods in use at the present time are not entirely satisfactory and considerable difficulty has been experienced by many analysts in securing concordant and entirely satisfactory results. Three different types of methods have been used in the past: (1) The absolute method of Dumas, which involves dry combustion and reduction of the gaseous products by a copper foil and measurement of the nitrogen formed; (2) the method of Will and Warrentrapp, in which the material is heated with soda lime

[1] Presented by I. K. Phelps.
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and the ammonia formed either titrated or weighed as ammonium platinic chloride; (3) the Kjeldahl method of converting the nitrogen present into ammonium sulfate by heating with strong sulfuric acid, then liberating the ammonia by means of strong soda solution, distilling and titrating.

The first two methods mentioned are the oldest. They are highly accurate, but very time-consuming and laborious. The Kjeldahl method is the one which is at present almost universally employed by food control officials and commercial chemists. This method, while simple in principle, requires considerable skill and close attention to minute details. The method itself was first described by Kjeldahl in 1883[1].

It seems that wet combustions, involving the use of alkali and potassium permanganate, were known prior to Kjeldahl's original article and it was by way of modification of the wet combustion process that Kjeldahl undertook the decomposition with permanganate under acid conditions. It should be emphasized that the use of an oxidizing agent was considered by Kjeldahl a vital step in the process and he attempted the use of phosphorus pentoxide and potassium dichromate, but considered permanganate preferable. The use of mercury or mercuric oxide was not mentioned by Kjeldahl in his original work.

It is extremely interesting to note that while potassium permanganate is a reagent upon which, to a large extent, the Kjeldahl method was originally based, the process has in the course of time been so modified that the desirability of its use is extremely doubtful.

Since Kjeldahl's article first appeared a number of investigations have been made by various chemists and an endless number of articles written on the subject. A great deal of good work has been done, although the results reported in some instances would indicate that the experimental work was not of an entirely satisfactory character. In these articles many modifications are suggested and the use of a large number of chemicals was investigated. However, only one or two investigators have introduced features which are of importance to the Kjeldahl method. Of these, an article written by J. W. Gunning in 1889[2] deserves first mention. Gunning found by the use of potassium sulfate that the time required for digestions may be very materially shortened.

The work of Wilfarth[3] should also receive recognition, since he first advocated the use of mercury.

There do not seem to have been published results of any investigation which take into consideration all the details of the method with the view to determining the causes for discrepancies in results, or the extent to which results may be affected by details in manipulation. It seemed

[1] *Compt. rend. trav. lab. Carlsberg*, 1883, **2**: 1; *Z. anal. Chem.*, 1883, **22**: 366.
[2] *Z. anal. Chem.*, 1889, **28**: 188.
[3] *Chem. Zentr.*, 1885, **56**: 17, 113.

desirable that such an investigation be undertaken in order that an analyst might understand fully where danger of inaccuracies lies and that he be advised as to the best means of avoiding these discrepancies. It also seemed desirable, in order that concordant and satisfactory results might be obtained, that minute details for carrying out the various steps in the Kjeldahl method be described.

The analyst who has had considerable experience with the Kjeldahl method is aware of the fact that more difficulty is experienced with some kinds of products than with others, and it seems that particular difficulty has been experienced with cottonseed meal. Cottonseed meal is an article which, at the present time, is of utmost importance and it is particularly desirable that the details of the method be so worked out as to yield accurate results on this commodity. It was with this material that the greater part of the work here recorded was done.

In conducting this investigation, the plan was to study first the influence of the various parts of the necessary apparatus. This work was carried out by the use of pure salts of ammonia. Subsequently, the details of the digestion were given careful consideration. This was accomplished in part on pure ammonium salts, and in part on samples of cottonseed meal, A and B. Thereafter, the method was studied with the samples indicated and the most desirable details decided upon and finally applied to products other than cottonseed meal.

The ammonium salts used as standards were C. P. products of Baker and Adamson. Careful examination in this laboratory showed them to be of a high degree of purity, fully within our requirements.

It will be noted that the greater part of the work was done upon the two samples, A and B, which were unknowns, so far as actual percentage of nitrogen was concerned. However, the number of determinations made was so great that there can be no doubt as to the actual nitrogen content: Sample A, 46.2 per cent; Sample B, 30.5 per cent.

The experimental work which was accomplished is described essentially in the tables. In order that they may be followed more readily, it seems desirable to explain them in a general manner at this time, and to tabulate and classify the headings.

The work is divided primarily into three parts. The first part includes Tables 1 to 10, and is a study of the effect of the use of various types of apparatus, and the various reagents which required attention. In this work only chemicals known to be pure were studied. Tables 1 to 5 are devoted to the operation of distillation. It may seem rather inconsistent to have commenced at the last of the operations involved in the determination of nitrogen by the Kjeldahl method, but it will be seen readily that by fixing the necessary details, step by step in the reverse order, the entire process may be worked out, while in starting with the first step there would always be an uncertainty as to whether observed difficul-

ties are caused at the point under consideration or at some later point. In the work recorded in Tables 6 to 10, the proper method of applying heat during the digestion with sulfuric acid is studied. The question of the use of an asbestos guard to confine the flame to that portion of the flask which contains the acid, is also taken up, also the necessity for the use of potassium sulfide.

In the second group of tables, which includes Tables 11 to 17, there are recorded the results obtained by the use of two samples of cottonseed meal. The purpose was to study the details for the successful decomposition of this substance.

Table 18 is a compilation of results obtained by the use of the details of the method which have been found to be most desirable.

Tables 19 to 23 give the results obtained on other common nitrogenous substances by this method.

The third group of tables, 24, 25, 26, represents an incidental study of the effect of nitrous fumes in the atmosphere surrounding the digestion, and of nitrates in the substance. This part of the work proved of unexpected interest.

To summarize, the subject matter may be arranged as follows:

APPARATUS AND REAGENTS.

DISTILLATION.

Table 1.—Efficiency of connecting bulbs.
Table 2.—Efficiency of distillation apparatus used throughout experiments.
Table 3.—Effect of varying quantities of standard acid and water in the receiving flasks.
Tables 4, 5.—Effect of quantity of distillate.

DIGESTION.

Table 6.—Effect of loss of sulfuric acid.
Table 7.—Effect of the use of a short-necked flask.
Table 8.—Effect of overheating.
Table 9.—Effect of use of asbestos guard.
Table 10.—Influence of the use of potassium sulfide.

DIGESTION OF COTTONSEED MEAL.

Table 11.—Effect of potassium permanganate.
Table 12.—Results of use of sulfuric acid alone.
Tables 13 and 14.—Influence of time on digestion.
Table 15.—Quantity of mercuric oxide.
Table 16.—Quantity of copper sulfate.
Table 17.—Quantity of sample used.
Table 18.—Series of results by use of proposed details.

RESULTS ON OTHER COMMON COMMODITIES.

Tables 19–23.

EFFECT OF NITRATES OR NITROUS FUMES.

Tables 24–26.

TABLE 1.

*Efficiency of connecting bulbs*.*

| TYPE OF BULB | 0.1 N ACID NEUTRALIZED | EQUIVALENT PROTEIN CALCULATED ON 2-GRAM CHARGE |
|---|---|---|
| | *cc.* | *per cent* |
| Plain bent tube, internal diameter ⅜ inch. | 2.2 | 0.96 |
| | 1.7 | 0.74 |
| | 1.9 | 0.83 |
| | 1.4 | 0.61 |
| Bent tube with plain bulb, diameter about 1⅝ inches. | 1.0 | 0.44 |
| | 0.6 | 0.26 |
| | 1.7 | 0.74 |
| | 0.6 | 0.26 |
| Kjeldahl connecting bulb, with internal inlet and outlet tubes bent in opposite directions. | 0.2 | 0.09 |
| | 0.3 | 0.14 |
| | 0.1 | 0.04 |

* Saturated sodium hydroxide, 100 cc., was diluted to 300 cc. and distilled into 0.1N acid.

TABLE 2.

*Efficiency of distillation apparatus used throughout experiments.*

| IN DISTILLATION FLASK | 0.1 N ACID REQUIRED TO TITRATE ABOUT 200 CC. OF DISTILLATE | EQUIVALENT PROTEIN CALCULATED ON 2-GRAM CHARGE |
|---|---|---|
| | *cc.* | *per cent* |
| 300 cc. of distilled water. | None | None |
| 300 cc. of distilled water and 1 cc. of saturated sodium hydroxide. | None | None |
| 250 cc. of distilled water and 50 cc. of saturated sodium hydroxide. | None | None |
| 200 cc. of distilled water and 100 cc. of saturated sodium hydroxide. | 0.1 | 0.04 |

The figures in Table 1 show that the Kjeldahl connecting bulb, as described, is much more efficient than other forms. Table 2 confirms this statement. This apparatus was used throughout this investigation.

TABLE 3.

*Effect of varying quantities of standard acid and water in the receiving flasks.*

| IN RECEIVING FLASK | AMMONIA FOUND | AMMONIA RECOVERED* |
|---|---|---|
| | *gram* | *per cent* |
| 5 cc. of water only | 0.1498 | 94.21 |
| 5 cc. of N hydrochloric acid | 0.1549 | 97.42 |
| 8 cc. of N hydrochloric acid | 0.1588 | 99.87 |
| 10 cc. of N hydrochloric acid | 0.1591 | 100.06 |
| 15 cc. of N hydrochloric acid | 0.1591 | 100.06 |
| 25 cc. of N hydrochloric acid | 0.1593 | 100.19 |
| 50 cc. of water only | 0.1540 | 96.85 |
| 5 cc. of N hydrochloric acid + 45 cc. of water | 0.1578 | 99.24 |
| 8 cc. of N hydrochloric acid + 42 cc. of water | 0.1585 | 99.68 |
| 10 cc. of N hydrochloric acid + 40 cc. of water | 0.1590 | 100.00 |
| 15 cc. of N hydrochloric acid + 35 cc. of water | 0.1590 | 100.00 |
| 25 cc. of N hydrochloric acid + 25 cc. of water | 0.1593 | 100.19 |

* Ammonium chloride (0.1590 gram of ammonia) was dissolved in 250 cc of water, 50 cc of saturated sodium hydroxide added and distilled

From Table 3 it will be seen that when enough acid is used in the receiving flask to neutralize 85.5 per cent of the ammonia distilled over, the amount retained was 99.87 and 99.68 per cent. Therefore, while it is advisable to use sufficient acid to neutralize all the ammonia to be distilled over, it is not absolutely necessary.

TABLE 4.

*Effect of quantity of distillate.*

| DISTILLATE | AMMONIA FOUND | AMMONIA RECOVERED* |
|---|---|---|
| *cc.* | *gram* | *per cent* |
| 15 | 0.1377 | 86.60 |
| 25 | 0.1563 | 98.30 |
| 50 | 0.1583 | 99.56 |
| 75 | 0.1588 | 99.87 |
| 100 | 0.1591 | 100.06 |
| | 0.1590 | 100.00 |
| 150 | 0.1588 | 99.87 |
| | 0.1590 | 100.00 |
| 200 | 0.1590 | 100.00 |
| | 0.1590 | 100.00 |
| 250 | 0.1590 | 100.00 |
| | 0.1590 | 100.00 |

* Ammonium chloride (0.1590 gram of ammonia), 250 cc of water and 50 cc. of saturated sodium hydroxide.

TABLE 5.

*Quantity of distillate necessary when very large amounts of nitrogen are present.*

| DISTILLATE | AMMONIA FOUND | AMMONIA RECOVERED* |
|---|---|---|
| *cc.* | *gram* | *per cent* |
| 50 | 0.3176 | 99.87 |
| | 0.3165 | 99.53 |
| 75 | 0.3169 | 99.65 |
| | 0.3181 | 100.03 |
| 100 | 0.3176 | 99.87 |
| | 0.3176 | 99.87 |
| 150 | 0.3177 | 99.90 |
| | 0.3182 | 100.06 |
| 200 | 0.3177 | 99.90 |

* Ammonium chloride (0.3180 gram of ammonia), 250 cc. of water and 50 cc. of saturated sodium hydroxide.

The results in Tables 4 and 5 show that practically all of the ammonia is distilled over with the first 75 cc. of distillate and all of it with 100 cc.

TABLE 6.

*Effect of the loss of sulfuric acid.*

| VOLUME IN DIGESTION FLASK ABOUT | NO POTASSIUM SULFATE USED | | 10 GRAMS OF POTASSIUM SULFATE USED | |
|---|---|---|---|---|
| | AMMONIA FOUND | AMMONIA RECOVERED* | AMMONIA FOUND | AMMONIA RECOVERED* |
| *cc.* | *gram* | *per cent* | *gram* | *per cent* |
| 5 | 0.1580 | 99.37 | 0.1573 | 98.92 |
| | 0.1581 | 99.43 | 0.1583 | 99.56 |
| 10 | 0.1590 | 100.00 | 0.1590 | 100.00 |
| | 0.1513 | 95.16 | 0.1591 | 100.06 |
| 20 | 0.1590 | 100.00 | 0.1590 | 100.00 |
| | 0.1587 | 99.82 | 0.1587 | 99.82 |

* Ammonium chloride (0.1590 gram of ammonia) and 25 cc. of sulfuric acid.

TABLE 7.

*Effect of the use of a short-necked flask (8 inches over all).*

| CONTENTS OF FLASK BOILED TO ABOUT | ASBESTOS GUARD NOT USED | | | | ASBESTOS GUARD USED | | | |
|---|---|---|---|---|---|---|---|---|
| | POTASSIUM SULFATE NOT USED | | 10 GRAMS OF POTASSIUM SULFATE USED | | POTASSIUM SULFATE NOT USED | | 10 GRAMS OF POTASSIUM SULFATE USED | |
| | AMMONIA FOUND | AMMONIA RECOVERED* | AMMONIA FOUND | AMMONIA RECOVERED* | AMMONIA FOUND | AMMONIA RECOVERED* | AMMONIA FOUND | AMMONIA RECOVERED* |
| *cc.* | *gram* | *per cent* | *gram* | *per cent* | *gram* | *per cent* | *gram* | *per cent* |
| 5 | 0.1445 | 90.88 | 0.0177 | 11.13 | 0.1585 | 99.68 | 0.1590 | 100.0 |
| 10 | 0.1367 | 85.97 | 0.1590 | 100.00 | 0.1590 | 100.00 | 0.1590 | 100.0 |
| | 0.1590 | 100.00 | ..... | ..... | ..... | ..... | ..... | ..... |
| 20 | 0.1564 | 98.36 | 0.1588 | 99.87 | ..... | ..... | ..... | ..... |

* Ammonium chloride (0.1590 gram of ammonia) and 25 cc. of sulfuric acid.

TABLE 8.

*Effect of overheating allowing strong flame to strike flask above acid.*

| CONTENTS OF FLASK BOILED TO ABOUT | NO POTASSIUM SULFATE USED | | 10 GRAMS OF POTASSIUM SULFATE USED | |
|---|---|---|---|---|
| | AMMONIA FOUND | AMMONIA RECOVERED* | AMMONIA FOUND | AMMONIA RECOVERED* |
| *cc.* | *gram* | *per cent* | *gram* | *per cent* |
| 5 | 0.1376 | 86.54 | 0.1566 | 98.49 |
| | 0.1301 | 81.82 | 0.1523 | 95.78 |
| | 0.1376 | 86.14 | 0.1557 | 97.92 |
| | [illegible] | | | |
| 10 | 0.1522 | 95.72 | 0.1552 | 97.62 |
| | 0.1500 | 94.35 | 0.1535 | 96.54 |
| | ..... | ..... | 0.1569 | 98.68 |

* Ammonium chloride (0.1590 gram of ammonia) and 20 cc. of sulfuric acid.

TABLE 9.

*Effect of a guard with a long-necked flask*.*

| CONTENTS OF FLASK BOILED TO ABOUT | NO POTASSIUM SULFATE USED | | 10 GRAMS OF POTASSIUM SULFATE USED | |
|---|---|---|---|---|
| | AMMONIA FOUND | AMMONIA RECOVERED† | AMMONIA FOUND | AMMONIA RECOVERED† |
| *cc.* | *gram* | *per cent* | *gram* | *per cent* |
| 5 | 0.1585 | 99.68 | 0.1588 | 99.87 |
| 10 | 0.1590 | 100.00 | 0.1590 | 100.00 |

* Twelve inches over-all.
† Ammonium chloride (0.1590 gram of ammonia) and 25 cc. of sulfuric acid.

From a study of Tables 6, 7, 8 and 9, the danger from allowing the flame to play too high upon the flask above the level of the liquid is clearly brought out. The volume of the liquid should be kept well above 10 cc. and the flame never allowed to touch the flask above the liquid. These tables demonstrate most emphatically the danger from this source and the necessity for some form of safety device. For this purpose, asbestos guards were used. These consisted of a one-fourth inch sheet of asbestos, with holes of such size that only that part of the flask which actually contains the liquid is exposed to the flame.

TABLE 10.

*Influence of the use of potassium sulfide.*

| DIGESTED WITH | DISTILLED | AMMONIA FOUND | AMMONIA RECOVERED* |
|---|---|---|---|
| | | *gram* | *per cent* |
| 0.7 gram of mercuric oxide | without potassium sulfide | 0.1572 | 98.86 |
| 0.7 gram of mercuric oxide | without potassium sulfide | 0.1399 | 87.99 |
| 0.7 gram of mercuric oxide | without potassium sulfide | 0.1459 | 91.76 |
| 1 gram of mercuric oxide+1 gram of copper sulfate | without potassium sulfide | 0.1445 | 90.88 |
| 1 gram of mercuric oxide+1 gram of copper sulfate | without potassium sulfide | 0.1459 | 91.76 |
| 1 gram of mercuric oxide+1 gram of copper sulfate | without potassium sulfide | 0.1367 | 85.97 |
| 1 gram of mercuric oxide+1 gram of copper sulfate | without potassium sulfide | 0.1418 | 89.18 |
| 0.7 gram of mercuric oxide | with potassium sulfide | 0.1591 | 100.06 |
| 0.7 gram of mercuric oxide | with potassium sulfide | 0.1593 | 100.19 |
| 0.7 gram of mercuric oxide | with permanganate and potassium sulfide | 0.1591 | 100.06 |
| 0.7 gram of mercuric oxide | with permanganate and potassium sulfide | 0.1590 | 100.00 |
| 0.7 gram of mercuric oxide | with permanganate and potassium sulfide | 0.1588 | 99.87 |
| 1 gram of mercuric oxide+1 gram of copper sulfate | with potassium sulfide | 0.1590 | 100.00 |
| 1 gram of mercuric oxide+1 gram of copper sulfate | with potassium sulfide | 0.1586 | 99.75 |
| 1 gram of mercuric oxide+1 gram of copper sulfate | with potassium sulfide | 0.1590 | 100.00 |
| 1 gram of mercuric oxide+1 gram of copper sulfate | with potassium sulfide | 0.1593 | 100.19 |
| 0.3 gram of copper sulfate | without potassium sulfide | 0.1591 | 100.06 |
| 0.3 gram of copper sulfate | without potassium sulfide | 0.1590 | 100.00 |
| 0.3 gram of copper sulfate+10 grams of potassium sulfate | without potassium sulfide | 0.1593 | 100.19 |
| 0.3 gram of copper sulfate+10 grams of potassium sulfate | without potassium sulfide | 0.1590 | 100.00 |
| 0.3 gram of copper sulfate+10 grams of potassium sulfate | with potassium sulfide | 0.1590 | 100.00 |
| 0.3 gram of copper sulfate+10 grams of potassium sulfate | with potassium sulfide | 0.1586 | 99.75 |

* Ammonium chloride (0.1590 gram of ammonia) and 25 cc. of sulfuric acid.

From Table 10 it will be seen that there is a loss of from 2 to 15 per cent of ammonia when mercury is used during digestion, if sulfide is not employed to precipitate the mercury before distillation. It is claimed that the property of mercury in holding back part of the ammonia is due to the formation of mercury-ammonium compounds. These are broken up by the potassium sulfide. It is also shown by this table that this property is not shared by copper, and that, therefore, when copper is used without mercury, the use of sulfide is unnecessary.

In the following experiments, whenever mercury was used in the digestion, potassium sulfide was used in the distillation. When no mercury was used, the sulfide was in each instance omitted.

### DIGESTION OF COTTONSEED MEAL.

It was believed desirable to use in this investigation a high protein and also a low protein meal and, in order to obtain this, a quantity of commercial meal was thoroughly air-dried and placed upon a 30-mesh sieve. The portion which passed through the sieve had the appearance of a high grade meal and is designated hereafter as Sample A. The portion which remained upon the sieve was carefully ground and also passed through the 30-mesh sieve. This portion had a brownish color and appeared to consist largely of hulls. It is designated as Sample B. Both portions were very thoroughly mixed and were then kept in large, air-tight, glass-stoppered bottles. Small portions were withdrawn from time to time for use in the investigation.

These samples contained the following percentages of protein:

Sample A 46.2 per cent;
Sample B 30.5 per cent.

In the following tables all results recorded for these two samples are in terms of percentage of protein, N×6.25.

TABLE 11.

*Effect of potassium permanganate.*

| DIGESTED* | POTASSIUM PERMANGANATE ADDED | SUBSEQUENT DIGESTION | PROTEIN | |
|---|---|---|---|---|
| | | | SAMPLE A | SAMPLE B |
| | | | *per cent* | *per cent* |
| Until straw colored | excess | none | 45.04 | 29.97 |
| Until straw colored | excess | 2 hours | 45.41 | 29.97 |
| Until straw colored | 0.2 gram at a time until 1 gram is added | ½ hour after each addition of potassium permanganate | 44.01<br>43.44 | 27.96<br>28.53 |
| Until clear | excess | 2 hours | 45.37 | 29.66 |
| Until clear | excess | 4 hours | 45.68 | 30.41 |
| Until clear | none | none | 45.37 | 30.58 |
| Until clear | excess | none | 45.24<br>45.82 | 30.06<br>30.58 |
| 2 hours after clear | none | none | 46.33 | 30.80 |
| 2 hours after clear | excess | none | 45.98 | 30.76 |
| 4 hours after clear | none | none | 46.29 | 30.71 |
| 4 hours after clear | excess | none | 46.37 | 30.71 |
| 6 hours after clear | none | none | 46.16 | 30.76 |
| 6 hours after clear | excess | none | 46.24 | 30.80 |
| 10 hours after clear | none | none | 46.46 | 30.63 |
| 10 hours after clear | excess | none | 46.20 | 30.67 |

* Digested 2 grams of sample with 30 cc. of sulfuric acid, 10 grams of potassium sulfate and 0.7 gram of mercuric oxide.

A study of Table 11 shows that potassium permanganate is of no value. In fact, the figures would indicate that there is a tendency for loss of ammonia, and the writers believe its use should be discontinued.

This question has recently been studied by Frear, Thomas and Edmiston[1], at the Agricultural Experiment Station, State College, Pa., who found, in working with fertilizers, that a distinct loss of nitrogen results, especially if the permanganate is added immediately after the flame under the flask is extinguished. If added later, the loss becomes smaller, but these authors in no instance record a gain. However, we are advised by Frear that they, and also D. C. Cochrane, have found indications of very slightly higher results by the use of this reagent in the examination of hay and other vegetable materials. However, this work has not been published, and at all events it would seem from our work that in general the danger attendant upon the use of potassium permanganate more than counterbalances the possible advantage which may be gained.

[1] *J. Assoc. Official Agr. Chemists*, 1919, 3: 220.

TABLE 12.

*Digestion with sulfuric acid alone*.*

| PROTEIN | |
|---|---|
| Sample A | Sample B |
| *per cent* | *per cent* |
| 45.06 | 30.19 |
| 45.19 | 30.01 |
| 45.15 | 30.08 |

* Period of digestion 17 hours with 35 cc. of sulfuric acid on a 2-gram samp e.

From Table 12, in connection with subsequent results, it will be seen that it apparently is impossible to get maximum results when sulfuric acid alone is used.

TAB

*Influence of time in connection with*

| TIME DIGESTED AFTER CLEAR | 5.0 GRAMS OF POTASSIUM SULFATE† | 10 GRAMS OF POTASSIUM SULFATE | 5.0 GRAMS OF SODIUM SULFATE‡ | 10 GRAMS OF SODIUM SULFATE | 0.7 GRAM OF MERCURIC OXIDE | 0.7 GRAM OF MERCURIC OXIDE 5 GRAMS OF POTASSIUM SULFATE | 0.7 GRAM OF MERCURIC OXIDE 10 GRAMS OF POTASSIUM SULFATE |
|---|---|---|---|---|---|---|---|
| *hours* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* |
| 0 | ..... | 45.44 | ..... | ..... | 45.50 | ..... | 45.37 |
| .. | ..... | 45.24 | ..... | ..... | 45.47 | ..... | 45.82 |
| 2 | ..... | 45.54 | ..... | ..... | 46.03 | 46.29 | 46.29 |
| .. | ..... | 45.72 | ..... | ..... | ..... | 46.29 | 46.16 |
| .. | ..... | ..... | ..... | ..... | ..... | ... | 46.29 |
| 3 | ..... | ..... | ..... | ..... | 45.63 | 46.24 | 46.46 |
| .. | ..... | ..... | ..... | ..... | 45.98 | 46.38 | 46.46 |
| 4 | ..... | 46.18 | ..... | 46.07 | 45.89 | 46.24 | 46.46 |
| .. | ..... | 45.76 | ..... | 45.85 | 46.03 | ..... | 46.29 |
| .. | ..... | ..... | ..... | ..... | ..... | ..... | 46.42 |
| 6 | 45.72 | 46.29 | 45.15 | 46.20 | 46.03 | ..... | 46.20 |
| . | 45.76 | 46.38 | 45.11 | 46.29 | . | . | 46.16 |
| .. | ..... | .. | 45.02 | . | . | . | 46.24 |
| .. | ..... | ..... | 45.11 | ..... | .. | .. | |
| 8 | ..... | 46.24 | ..... | . | | ..... | 46.12 |
| .. | ..... | ..... | ..... | ..... | . | . | . |
| 10 | ..... | 46.42 | ..... | ..... | 46.21 | ..... | 46.20 |
| .. | ..... | ..... | ..... | ..... | .. | .. | 46.46 |
| .. | ..... | ..... | ..... | . | | .. | 46.24 |
| 20 | . | ..... | ..... | ..... | . | . | 46.37 |
| .. | ..... | ..... | ..... | ..... | . | ..... | 46.24 |

* Sample A, 2 grams with 30 cc of sulfuric acid.
† Clear after 4 hours' digestion.
‡ Not entirely clear after 6 hours' digestion.

LE 13.

*various reagents in the determination of protein*.*

| 0.7 GRAM OF MERCURIC OXIDE 5 GRAMS OF SODIUM SULFATE | 0.7 GRAM OF MERCURIC OXIDE 10 GRAMS OF SODIUM SULFATE‡ | 0.5 GRAM OF COPPER SULFATE | 0.5 GRAM OF COPPER SULFATE 5 GRAMS OF POTASSIUM SULFATE | 0.5 GRAM OF COPPER SULFATE 10 GRAMS OF POTASSIUM SULFATE | 0.5 GRAM OF COPPER SULFATE 5 GRAMS OF SODIUM SULFATE | 0.5 GRAM OF COPPER SULFATE 10 GRAMS OF SODIUM SULFATE | 1 GRAM OF MERCURIC OXIDE 1 GRAM OF COPPER SULFATE 15 GRAMS OF POTASSIUM SULFATE |
|---|---|---|---|---|---|---|---|
| *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* |
| ..... | 45.98 | 45.06 | ..... | 45.24 | ..... | ..... | 45.89 |
| ..... | 45.89 | ..... | ..... | 45.06 | ..... | ..... | ..... |
| 46.24 | 46.20 | 45.50 | ..... | 45.68 | ..... | ..... | 46.33 |
| 46.29 | 46.24 | ..... | ..... | 45.63 | ..... | ..... | ..... |
| ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| 46.33 | 46.38 | ..... | ..... | ..... | ..... | ..... | ..... |
| 46.24 | 46.29 | ..... | ..... | ..... | ..... | ..... | ..... |
| 46.29 | ..... | 45.37 | ..... | 46.16 | 46.24 | ..... | 46.33 |
| 46.29 | ..... | ..... | ..... | 46.07 | 45.98 | ..... | ..... |
| 46.24 | ..... | ..... | ..... | 46.11 | ..... | ..... | ..... |
| ..... | ..... | 45.24 | 45.94 | 46.20 | 45.85 | 46.24 | 46.29 |
| ..... | ..... | ..... | 45.76 | 46.33 | 45.63 | 46.07 | ..... |
| ..... | ..... | ..... | ..... | 46.29 | ..... | 46.38 | ..... |
| ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| ..... | ..... | ..... | 46.20 | 46.24 | 46.20 | ..... | ..... |
| ..... | ..... | ..... | 46.11 | 46.42 | ..... | ..... | ..... |
| ..... | ..... | 45.72 | ..... | 46.33 | ..... | ..... | 46.24 |
| ..... | ..... | ..... | ..... | 46.39 | ..... | ..... | ..... |
| ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| ..... | ..... | ..... | ..... | 46.33 | ..... | ..... | ..... |
| ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |

TAB

*Influence of time in connection with various*

| TIME DIGESTED AFTER CLEAR | 10 GRAMS OF POTASSIUM SULFATE | 10 GRAMS OF SODIUM SULFATE | 0.7 GRAM OF MERCURIC OXIDE | 0.7 GRAM OF MERCURIC OXIDE 5 GRAMS OF POTASSIUM SULFATE | 0.7 GRAM OF MERCURIC OXIDE 10 GRAMS OF POTASSIUM SULFATE | 0.7 GRAM OF MERCURIC OXIDE 5 GRAMS OF SODIUM SULFATE |
|---|---|---|---|---|---|---|
| *hours* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* |
| 0 | 30.10 | ..... | 30.58 | ..... | 30.58 | .... |
| .. | 29.75 | ..... | ..... | ..... | 30.58 | ..... |
| 2 | 30.45 | ..... | 30.53 | 30.71 | 30.76 | 30.63 |
| .. | 30.14 | ..... | ..... | 30.63 | 30.76 | 30.63 |
| 3 | ..... | ..... | ..... | 30.58 | 30.63 | 30.67 |
| .. | ..... | ..... | ... | 30.54 | 30.63 | 30.67 |
| 4 | 30.63 | 30.36 | 30.71 | 30.67 | 30.63 | 30.63 |
| .. | 30.32 | 30.28 | ..... | 30.67 | 30.71 | 30.58 |
| .. | ..... | ..... | ..... | ..... | ..... | ..... |
| 6 | 30.67 | 30.63 | 30.49 | .... | 30.80 | ..... |
| .. | 30.49 | ..... | ..... | ..... | 30.80 | ..... |
| .. | ..... | ..... | ..... | ..... | ..... | ..... |
| 8 | 30.67 | ..... | ..... | ..... | 30.58 | ..... |
| .. | ..... | ..... | ..... | ..... | 30.89 | ..... |
| 10 | 30.71 | ..... | 30.58 | ..... | 30.67 | ..... |
| .. | 30.62 | ..... | ..... | ..... | 30.67 | ..... |
| 20 | ..... | ..... | ..... | ..... | 30.71 | ..... |
| .. | ..... | ..... | ..... | ..... | 30.67 | ..... |

* Sample B, 2 grams, with 30 cc. of sulfuric acid.

From a study of Tables 13 and 14, it will be seen that when 10 grams of either potassium or sodium sulfate were used, maximum results were reached in 6 hours, but with 5 grams an excessive time was required to clear up the solution and maximum results were not reached in 6 hours' additional digestion. With mercury alone the results were not satisfactory since approximately 10 hours were required to complete the digestion. However, when both mercury and potassium sulfate were used maximum results were obtained with 2 hours' digestion after clearing. It will be noted that there appears to be little difference between 5 grams and 10 grams of sulfate when used with mercury. When copper sulfate alone is used, maximum results were not reached even after 10 hours' digestion. But with both copper sulfate and potassium sulfate maximum results were reached after 6 hours. Mercury is much more efficient than copper, as when mercury alone was used maximum results were reached in 10 hours, while with copper alone this was not the case. This conclusion is

LE 14.

*reagents in the determination of protein*.*

| 0.7 GRAM OF MERCURIC OXIDE 10 GRAMS OF SODIUM SULFATE | 0.5 GRAM OF COPPER SULFATE | 0.5 GRAM OF COPPER SULFATE 5 GRAMS OF POTASSIUM SULFATE | 0.5 GRAM OF COPPER SULFATE 10 GRAMS OF POTASSIUM SULFATE | 0.5 GRAM OF COPPER SULFATE 5 GRAMS OF SODIUM SULFATE | 0.5 GRAM OF COPPER SULFATE 10 GRAMS OF SODIUM SULFATE | 1 GRAM OF MERCURIC OXIDE 1 GRAM OF COPPER SULFATE 15 GRAMS OF POTASSIUM SULFATE |
|---|---|---|---|---|---|---|
| *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* |
| 30.28 | 29.75 | ..... | 29.97 | ..... | ..... | 30.50 |
| 30.01 | ..... | ..... | 29.75 | ..... | ..... | ..... |
| 30.59 | 29.97 | ..... | 30.19 | ..... | ..... | 30.71 |
| 30.67 | ..... | ..... | 30.19 | ..... | ..... | ..... |
| 30.54 | ..... | ..... | ..... | ..... | ..... | ..... |
| 30.48 | ..... | ..... | ..... | ..... | ..... | ..... |
| ..... | 30.10 | ..... | 30.67 | ..... | ..... | 30.75 |
| ..... | ..... | ..... | 30.71 | ..... | ..... | ..... |
| ..... | ..... | ..... | 30.67 | ..... | ..... | ..... |
| ..... | 29.97 | 30.22 | 30.71 | 30.01 | 30.71 | 30.71 |
| ..... | ..... | 30.54 | 30.80 | 30.32 | ..... | ..... |
| ..... | ..... | ..... | 30.68 | ..... | ..... | ..... |
| ..... | ..... | 30.63 | 30.49 | 30.41 | ..... | ..... |
| ..... | ..... | 30.49 | 30.63 | 30.58 | ..... | ..... |
| ..... | 29.27 | ..... | 30.63 | ..... | ..... | 30.71 |
| ..... | ..... | ..... | 30.89 | ..... | ..... | ..... |
| ..... | ..... | ..... | 30.76 | ..... | ..... | ..... |
| ..... | ..... | ..... | ..... | ..... | ..... | ..... |

also borne out by the fact that with mercury and potassium sulfate maximum results are obtained in from 2 to 3 hours, while with copper sulfate and potassium sulfate they were reached in 5 to 6 hours. Then again, a longer time is required to clear up the solution when copper sulfate is used than when mercury is used. It would appear that potassium sulfate is a little more efficient than sodium sulfate, as with 5 grams of the former the solution was clear in 4 hours, while with 5 grams of sodium sulfate the solution was not entirely clear after 6 hours. From the results obtained the conclusion must be drawn that the most efficient combination is mercury and 5 to 10 grams of either potassium or sodium sulfate. One objection sometimes raised to the use of mercury is its greater cost over copper sulfate, but it would seem that this objection is completely offset by the time consumed in the case of the latter. There is no advantage in the combination of mercury and copper sulfate as directed in the Kjeldahl-Gunning-Arnold method.

TABLE 15.

*Effect of mercuric oxide* used in the determination of protein.*

| MERCURIC OXIDE USED | TIME REQUIRED TO CLEAR | SAMPLE A | | | SAMPLE B | | |
|---|---|---|---|---|---|---|---|
| | | Hours digested after clear | | | Hours digested after clear | | |
| | | 2 | 3 | 4 | 2 | 3 | 4 |
| *gram* | *hours* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* |
| 0.0 | 2 | 45.54 | ..... | 45.76 | 30.45 | ..... | 30.63 |
| | | 45.72 | ..... | 46.18 | 30.14 | ..... | 30.32 |
| 0.1 | 1½ | 45.68 | 45.89 | 46.29 | 30.36 | 30.19 | 30.63 |
| | | 45.94 | 46.03 | 46.20 | 30.45 | ..... | 30.71 |
| 0.2 | 1½ | 45.98 | 46.20 | 46.16 | 30.49 | 30.54 | 30.63 |
| | | 45.76 | ..... | 46.16 | 30.41 | ..... | 30.58 |
| | | ..... | ..... | 46.29 | ..... | ..... | 30.63 |
| 0.3 | 1 | 45.94 | 46.20 | 46.29 | 30.58 | 30.54 | 30.76 |
| | | 46.03 | 46·29 | ..... | 30.32 | ..... | ..... |
| 0.5 | 1 | 46.20 | 46.24 | 46.33 | 30.49 | 30.63 | 30.63 |
| | | 46.29 | 46.33 | ..... | 30.45 | 30.65 | ..... |
| 0.7 | 1 | 46.29 | 46.33 | 46.24 | 30.76 | 30.67 | 30.59 |
| | | 46.33 | 46.33 | 46.29 | 30.76 | 30.71 | 30.80 |
| | | ..... | ..... | 46.29 | 30.58 | 30.76 | 30.71 |
| 1.0 | 1 | 46.16 | 46.16 | 46.38 | 30.55 | 30.49 | 30.71 |
| | | 46.38 | ..... | ..... | 30.55 | ..... | ..... |
| | | ..... | ..... | ..... | 30.55 | ..... | ..... |

* Digested 2 grams of sample with 30 cc. of sulfuric acid and 10 grams of potassium sulfate.

The figures in Table 15 show that from 0.5 to 0.7 gram of mercuric oxide is the proper amount to use. In fact, 0.3 gram gives maximum results with 3 hours' digestion after clear, while 0.5 gram or over gives the maximum in 2 hours. There certainly is nothing to be gained by the use of more than 0.7 gram.

TABLE 16.

*Effect of copper sulfate* used in the determination of protein.*

| COPPER SULFATE USED | TIME REQUIRED TO CLEAR | SAMPLE A | | | SAMPLE B | | |
|---|---|---|---|---|---|---|---|
| | | Hours digested after clear | | | Hours digested after clear | | |
| | | 5 | 6 | 7 | 5 | 6 | 7 |
| *gram* | *hours* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* |
| 0.1 | 1¾ | 46.38 | 46.29 | 46.33 | 30.42 | 30.67 | 30.63 |
| | | 46.16 | 46.24 | 46.16 | 30.80 | 30.71 | 30.71 |
| | | ..... | 46.23 | ..... | ..... | ..... | ..... |
| 0.3 | 1¼ | 45.41 | 46.24 | 46.24 | 30.49 | 30.42 | 30.54 |
| | | 46.20 | 46.20 | 46.20 | 30.58 | 30.63 | 30.80 |
| | | ..... | ..... | 46.24 | .... | 30.58 | .. .. |
| 0.5 | 1¼ | 45.37 | 45.85 | 46.16 | 30.54 | 30.63 | 30.49 |
| | | 45.54 | 46.33 | 46.33 | 30.63 | 30.54 | 30.71 |
| | | 46.38 | 46.33 | 46.29 | 30.63 | 30.67 | 30.76 |
| | | 46.38 | 46.20 | 46.33 | . . . | 30.71 | 30.71 |
| | | .. .. | 46.16 | 46.24 | ..... | 30.71 | 30.54 |
| 1.0 | 1¼ | 45.98 | 46.38 | 46.16 | 30.53 | 30.63 | 30.67 |
| | | 46.16 | 46.38 | 46.24 | 30.45 | ..... | ..... |

* Digested 2 grams of sample with 30 cc of sulfuric acid and 10 grams of potassium sulfate.

From Table 16 it will be seen that the quantity of copper sulfate used has very little influence.

TABLE 17.

*Effect of quantity of sample* used in the determination of protein.*

| TIME DIGESTED AFTER CLEAR | 0.5-GRAM SAMPLE | | 1-GRAM SAMPLE | | 2-GRAM SAMPLE | |
|---|---|---|---|---|---|---|
| | Sample A | Sample B | Sample A | Sample B | Sample A | Sample B |
| *hours* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* |
| 1 | 46.28 | ... | 46.11 | 30.63 | .. | .... |
| | 46.20 | .... | 46.11 | 30.71 | . | .... |
| 2 | 46.38 | 30.28 | 46.38 | 30.63 | 46.29 | 30.76 |
| | 46.03 | 30.45 | 46.29 | 30.71 | 46.33 | 30.58 |
| 3 | 46.73 | 30.45 | 46.38 | 30.71 | 46.33 | 30.67 |
| | 46.55 | .... | 46.29 | 30.71 | 46.33 | 30.71 |

* Digested with 30 cc. of sulfuric acid, 0.7 gram of mercuric oxide and 10 grams of potassium sulfate.

The figures in Table 17 were obtained by using 0.5-, 1- and 2-gram samples. Maximum results were reached with a slightly shorter time of digestion with the smaller amount of sample, but much greater diffi-

culty was experienced in getting concordant results. This is undoubtedly due to the fact that the smaller the sample weighed, the greater the difficulty of obtaining a representative sample, and the multiplication of slight unavoidable errors. Everything considered, it seems that a 2-gram sample of cottonseed meal and similar substances is preferable to other amounts.

TABLE 18.

*Protein obtained by using the details found to be most desirable*.*

| 0.7 GRAM OF MERCURIC OXIDE DIGESTED 3 HOURS AFTER CLEAR | | 0.3 GRAM OF COPPER SULFATE DIGESTED 6 HOURS AFTER CLEAR | |
|---|---|---|---|
| Sample A | Sample B | Sample A | Sample B |
| *per cent* | *per cent* | *per cent* | *per cent* |
| 46.24 | 30.58 | 46.11 | 30.49 |
| 46.24 | 30.54 | 46.20 | 30.49 |
| 46.24 | 30.54 | 46.16 | 30.58 |
| 46.16 | 30.41 | 46.07 | 30.58 |
| 46.03 | 30.49 | 46.20 | 30.54 |
| 46.11 | 30.51 | 46.03 | 30.58 |
| 46.24 | 30.41 | 46.11 | 30.62 |
| 46.03 | 30.41 | 45.94 | 30.45 |
| 46.20 | 30.58 | 45.94 | 30.58 |
| 46.16 | 30.41 | 46.11 | 30.36 |
| 46.24 | 30.45 | 46.24 | 30.36 |
| 46.03 | 30.45 | 46.11 | 30.41 |
| Av.....46.16 | 30.48 | 46.09 | 30.50 |
| Min....46.03 | 30.41 | 45.94 | 30.41 |
| Max....46.24 | 30.58 | 46.24 | 30.62 |

* Digested 2 grams of sample with 30 cc. of sulfuric acid and 10 grams of potassium sulfate.

## METHOD.

After due consideration of the figures given in these tables, together with the experience gained during this investigation, the writers consider the following details to be such that if carefully followed in every respect no difficulty should be experienced in getting satisfactory results when working upon cottonseed meal or similar substances by individual or different analysts:

Grind the sample to such a fineness that it will pass through a 30-mesh sieve, or a sieve having round 1 mm. openings. Place 2 grams of this thoroughly mixed sample in a 500 cc. digestion flask, add 0.5–0.7 gram of mercuric oxide or its equivalent of metallic mercury, 5–10 grams of potassium or sodium sulfate and shake the flask until the contents are well mixed. Add 30 cc. of sulfuric acid and again shake until the acid and dry material in the flask are perfectly homogeneous. This mixture should be practically free from lumps. Place the flask in an inclined position, using a guard having a hole for the flask of such a size that the flask can never be exposed to the bare flame

above the acid at any time during digestion, and heat with a very low flame until frothing ceases. Gradually increase the flame until the liquid boils briskly, using a flame with the air so regulated that it gives a sharp-pointed blue flame. Continue this heat until the solution is entirely clear. This should require 1–1½ hours. Decrease the heat until the liquid boils gently and continue this digestion for 3 hours. Allow to cool, add 230 cc. of water and 20 cc. of 4% potassium sulfide. Shake, and add an excess of saturated sodium hydroxide solution (50–60 cc.), allowing it to run down the side of the flask so that it does not mix with the acid solution. Add a few pieces of granulated zinc and connect the flask with the distillation apparatus. This apparatus must be equipped with the most efficient form of Kjeldahl connecting bulb, inserted between distillation flask and condenser. Mix the contents by shaking, and distil about 150 cc. into very accurately measured standard acid, using an excess of acid necessary to hold all the ammonia.

If it is desired to use copper sulfate in place of mercuric oxide, substitute 0.3–0.5 gram of copper sulfate for the mercury and digest for 6 hours after the solution becomes clear and omit the potassium sulfide.

## EXAMINATION OF OTHER PRODUCTS.

To ascertain the length of time necessary to digest in order to obtain the maximum results in various other substances, determinations were made on wheat flour, powdered milk, gelatin, egg-albumin, and tankage.

TABLE 19.

*Time required for digestion of flour.*

| 2-GRAM SAMPLE, 30 CC. OF SULFURIC ACID, 10 GRAMS OF POTASSIUM SULFATE | MERCURIC OXIDE USED | COPPER SULFATE USED |
|---|---|---|
| Time digested after clear | Protein (N X 5.70) | |
| *hours* | *per cent* | *per cent* |
| ½ | 10.09 | ..... |
| 1 | 10.29 | ..... |
| | 10.29 | ..... |
| 2 | 10.20 | ..... |
| | 10.13 | 10.17 |
| 3 | 10.25 | 10.37 |
| | 10.20 | 10.21 |
| 4 | ..... | 10.29 |
| | ..... | 10.21 |
| 5 | ..... | 10.29 |
| | ..... | 10.21 |

TABLE 20.

*Time required for digestion of powdered milk.*

| 2-GRAM SAMPLE, 30 CC. OF SULFURIC ACID, 10 GRAMS OF POTASSIUM SULFATE | MERCURIC OXIDE USED | COPPER SULFATE USED |
|---|---|---|
| Time digested after clear | Protein (N X 6.38) | |
| *hours* | *per cent* | *per cent* |
| ½ | 32.83 | ..... |
| | 32.83 | .... |
| 1 | 32.87 | ..... |
| 2 | 32.83 | 32.38 |
| | 32.83 | ..... |
| 3 | 32.87 | 32.87 |
| | 32.69 | 32.83 |
| 5 | ..... | 32.65 |
| | ..... | 32.74 |
| 6 | ..... | 32.74 |
| | ..... | 32.83 |

TABLE 21.

*Time required for digestion of gelatin.*

| 2-GRAM SAMPLE, 30 CC. OF SULFURIC ACID, 10 GRAMS OF POTASSIUM SULFATE | MERCURIC OXIDE USED | COPPER SULFATE USED |
|---|---|---|
| Time digested after clear | Protein (N X 5.55) | |
| *hours* | *per cent* | *per cent* |
| 2 | 85.39 | ..... |
| | 85.35 | ..... |
| 3 | 85.35 | ..... |
| 4 | 85.43 | ..... |
| | 85.47 | ..... |
| 5 | ..... | 85.04 |
| | ..... | 85.09 |
| 6 | ..... | 85.47 |
| | ..... | 85.43 |
| 7 | ..... | 85.47 |
| | ..... | 85.51 |

TABLE 22.

*Time required for digestion of egg-albumin.*

| 2-GRAM SAMPLE, 30 CC. OF SULFURIC ACID, 10 GRAMS OF POTASSIUM SULFATE | MERCURIC OXIDE USED | COPPER SULFATE USED |
|---|---|---|
| Time digested after clear | Protein (N X 6.25) | |
| *hours* | *per cent* | *per cent* |
| ½ | 76.96 | ..... |
| | 76.65 | ..... |
| 1 | 77.35 | ..... |
| | 77.44 | ..... |
| 2 | 77.35 | ..... |
| | 77.35 | ..... |
| 3 | 77.44 | ..... |
| | 77.35 | ..... |
| 4 | ..... | 76.91 |
| | ..... | 76.83 |
| 5 | ..... | 77.39 |
| | ..... | 77.09 |
| 6 | ..... | 77.13 |
| | ..... | 77.04 |
| 7 | ..... | 77.35 |
| | ..... | 77.31 |

TABLE 23.

*Time required for digestion of tankage.*

| 2-GRAM SAMPLE, 30 CC. OF SULFURIC ACID, 10 GRAMS OF POTASSIUM SULFATE | MERCURIC OXIDE USED | COPPER SULFATE USED |
|---|---|---|
| Time digested after clear | Protein (N X 6.25) | |
| *hours* | *per cent* | *per cent* |
| 3 | 49.53 | ..... |
| | 49.44 | ..... |
| 4 | 49.61 | ..... |
| | 49.64 | ..... |
| 5 | 49.74 | ..... |
| | 49.70 | ..... |
| 6 | ..... | 48.83 |
| | ..... | 48.74 |
| 7 | ..... | 49.25 |
| | ..... | 49.39 |
| 8 | ..... | 49.66 |
| | ..... | 49.74 |
| 9 | ..... | 49.88 |
| | ..... | 49.83 |

From Tables 19 to 23, it will be seen that 1-hour digestion with mercury and 2 hours with copper sulfate is sufficient in the case of flour, ½ hour and 2 hours in the case of powdered milk, 2 and 6 hours for gelatin, 1 and 5 hours in the case of egg-albumin, and 4 and 8 hours for tankage.

### EFFECT OF NITROUS FUMES.

So far as known, there has never been anything published regarding the effect of nitrates or nitric oxides introduced at different stages during the digestion. For the purpose of studying this question, the following experiments were conducted.

TABLE 24.

*Effect of introduction of nitrates or nitric oxides at beginning of digestion*.*

| AMMONIA AS AMMONIUM SULFATE ADDED | POTASSIUM NITRATE EQUIVALENT TO AMMONIA ADDED | AMMONIA RECOVERED | AMMONIA RECOVERED |
|---|---|---|---|
| *gram* | *gram* | *gram* | *per cent* |
| 0.1688 | 0.102 | 0.2002 | 118.6 |
| 0.1688 | 0.051 | 0.1918 | 112.5 |
| 0.0844 | 0.102 | 0.1214 | 143.6 |
| 0.1688 | 0.034 | 0.1848 | 109.4 |
| 0.1688 | 0.017 | 0.1783 | 105.6 |
| 0.1688 | 0.010 | 0.1722 | 102.0 |
| 0.1688 | 0.005 | 0.1746 | 103.4 |
| 0.1688 | 0.102 | 0.2006 | 118.8 |
| 0.1688 | 0.051 | 0.1918 | 112.5 |
| Ammonia from cottonseed meal: | | | |
| 0.1795† | 0.0336 | 0.2077 | 115.7 |
| 0.1185‡ | 0.0336 | 0.1547 | 130.5 |

* Digest 30 cc. of sulfuric acid with 0.7 gram of mercuric oxide or 0.3 gram of copper sulfate, 10 grams of potassium sulfate, and 1 gram of sugar.

TABLE 25.

*Effect of nitrates or nitric oxides introduced after solution is clear*.*

| AMMONIA AS AMMONIUM SULFATE ADDED | POTASSIUM NITRATE ADDED | AMMONIA RECOVERED | AMMONIA RECOVERED |
|---|---|---|---|
| *gram* | *gram* | *gram* | *per cent* |
| 0.1688 | 0.1 | 0.1547 | 91.1 |
| 0.1688 | 0.1 | 0.1527 | 90.5 |
| 0.1688 | 1.0† | 0.0753 | 44.6 |
| 0.1688 | 1.0† | 0.0712 | 42.2 |
| 0.1688 | 1.0† | 0.0615 | 36.5 |
| 0.1688 | 1.0† | 0.0707 | 41.9 |
| 2 grams of cottonseed meal, "A" | 1.0† | 0.1224 | 68.2 |
| 2 grams of cottonseed meal, "B" | 1.0† | 0.1022 | 86.2 |

* Digest 30 cc. of sulfuric acid with 0.7 gram of mercuric oxide or 0.3 gram of copper sulfate and 10 grams of potassium sulfate.

† Added 0.1 gram at intervals during digestion.

TABLE 26.

*Digestions in presence of nitric acid fumes.*

| SAMPLE A | | | SAMPLE B | | |
|---|---|---|---|---|---|
| Protein in sample | Protein found | Total protein lost | Protein in sample | Protein found | Total protein lost |
| *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* |
| 46.20 | 43.93 | 4.91 | 30.50 | 26.56 | 12.91 |
| .... | 43.44 | 5.97 | .... | 25.64 | 15.93 |
| .... | 37.98 | 17.79 | .... | 20.30 | 33.44 |
| .... | 40.12 | 13.16 | .... | 21.91 | 28.16 |
| .... | 32.20 | 30.30 | .... | 23.32 | 23.86 |
| .... | 35.35 | 23.48 | .... | 24.50 | 19.67 |
| .... | 31.06 | 32.77 | .... | .... | .... |
| .... | 28.57 | 38.16 | .... | .... | .... |

It seems quite natural that the presence of nitrates in a sample consisting largely of organic material, would, by the Kjeldahl method, yield a higher result than would be obtained without the nitrates. This would be expected, since the organic matter present would probably bring about at least a partial reduction of the nitric nitrogen to ammonia. This is well understood, and it is also known that the reduction is not complete, but that there is a loss of nitric nitrogen. Table 24 shows this definitely.

The question now is, what will be the result of adding nitric nitrogen after the organic matter is fully decomposed? To answer this, the experiments recorded in Table 25 were undertaken. It will be seen that not only is the nitric nitrogen lost entirely, but there is, in addition, a considerable loss of the organic or ammoniacal nitrogen, amounting in one instance to as much as 58 per cent. The reaction may be essentially $10\,NH_3 + 3\,N_2O_3 = 8N_2 + 15\,H_2O$. Probably, especially in the presence of organic matter, the reaction is, in fact, far more complex.

The next question was the possible effect of conducting nitrogen determinations in the presence of nitrous fumes. The results recorded in Table 26 were, accordingly, obtained in a hood in which there were present large quantities of red oxides of nitrogen. Contrary to expectation, these fumes could be observed travelling down the lower side of the neck of the flask, notwithstanding the fact that fumes were being given off in the digestion reaction and could be seen plainly escaping along the upper side of the neck of the flask. The results show a remarkable loss of nitrogen, amounting to as much as 38 per cent.

This will show the extreme importance of eliminating and carefully guarding against the presence of any nitrous fumes. No nitric acid should ever be used in a hood where nitrogen digestions are to be made, as these fumes have a tendency to persist, even in spite of a strong draft. This

is especially true if a lead tube is used to carry off the sulfuric acid fumes. Usually, the necks of the digestion flasks pass through openings into this lead tube, and sulfuric acid collects in the bottom. If, later, nitric fumes are liberated in the hood, the condensed sulfuric acid will tend to retain some of these fumes. During subsequent nitrogen determinations the heat will liberate these and they will enter the necks of the flasks and cause discordant and erroneous results.

## SUMMARY.

The interesting and important points developed in the investigation are:

(1) The use of an efficient distillation bulb is important.

(2) The use of a guard in digestion is essential.

(3) The use of potassium permanganate is at least unnecessary.

(4) The reagents, in addition to sulfuric acid, which are most economical of time are combined potassium, or sodium sulfate, mercuric oxide and potassium sulfide.

(5) Copper sulfate may be used in place of mercuric oxide and potassium sulfide, but the time of digestion must be lengthened.

(6) The time of digestion after clearing is of prime importance, and should be determined for each kind of substance to be examined. In most instances 3 hours is sufficient.

(7) Nitric acid should not be used in a hood in which it is the intention to make nitrogen digestions at a future time.

No referee on potash was appointed and no special report on this subject was presented.

# REPORT ON POTASH AVAILABILITY.

By A. G. McCall (Agricultural Experiment Station, College Park, Md.), *Referee.*

The investigation of the availability of the potassium of greensand composted with manure and sulfur has been continued during the year at the Maryland Agricultural Experiment Station. Reports from other Agricultural Experiment Stations show that the official method for the determination of potash does not give full credit for the available potash found in the so-called treater dust and wood ashes. Haskins[1] called attention to this and suggested the advisability of devising some modi-

[1] *J. Assoc. Official Agr. Chemists*, 1920, 4: 82.

fication of the official method that would include not only the potash dissolved by hot water, but also that which would be readily broken down in the soil and thus become available to the growing plants. Before any definite conclusions can be reached, it will be necessary to make vegetation tests with some of these materials to see if the potash that is lightly locked up as basic compounds may not become readily available to growing plants when incorporated with the soil. While this question is not so important as it was during the war period when our European supply of potash was cut off, your referee is of the opinion that the association should interest itself in this matter with a view to obtaining data upon which some definite action may be taken.

## RECOMMENDATIONS.

It is recommended—

(1) That the work on the availability of potash in composts, treater dust and wood ashes be continued.

(2) That a general referee on potash be appointed to continue the study of the perchlorate method with special reference to the moist combustion process[1], and to make a further study of the effect of using stronger alcohol for the first washings in the official Lindo-Gladding method with special reference to the presence of sodium salts.

# POT CULTURE TESTS ON THE AVAILABILITY OF POTASSIUM FROM GREENSAND COMPOSTS.

By A. M. Smith (Agricultural Experiment Station, College Park, Md.).

Glauconite, commonly known as greensand, consists chiefly of the hydrous silicate of iron, aluminium, and potassium. Because extensive deposits of this mineral exist as outcrops and subsoil in New Jersey, Maryland and Virginia, it was thought advisable, during the period of potash shortage, to investigate the possible use of greensand as a fertilizer.

Previous work at the Maryland Agricultural Experiment Station[2] showed that composting greensand with sulfur and organic matter for a period of from 15 to 23 weeks changed a considerable part of the insoluble potassium to a form soluble in water. In this paper it is desired to report some results of pot culture tests on the availability of the potassium from these composts, and to present other points of chemical interest developed during the progress of the investigation.

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[1] *J. Assoc. Official Agr. Chemists*, 1921, 4: 373.
[2] *Ibid.*, 375.

Barley was grown to maturity in a series of glazed pots, each pot having 2800 grams of Collington sandy loam containing 0.82 per cent of potassium. After germinating the seeds on a cheesecloth screen in contact with a weak germinating solution, four seedlings were transplanted to each pot.

The treatments were planned with the following objects in view:

(1) To compare the availability and effectiveness of equal amounts of potassium from potassium sulfate, composted greensand, and untreated greensand.

(2) To compare composted greensand with the uncomposted materials in the same amounts and proportion, with and without the use of lime.

Each treatment was in duplicate. Each pot, including the controls, received 0.5 gram of ammonium sulfate and 0.5 gram of monocalcium phosphate. The potassium treatments are given in Table 1, the amount in each case being equal to that contained in 0.5 gram of potassium sulfate.

TABLE 1.

*Results of pot culture tests.*

| POT NUMBER | TREATMENT | CROP YIELD* | SOIL ACIDITY* |
|---|---|---|---|
| | | Dry weight of tops | Calcium carbonate by Veitch's method |
| | | *gram* | *p. p. m.* |
| 1 and 2 | Control. | 12.9 | 1600 |
| 3 and 4 | Greensand, 4.04 grams. | 13.4 | 2350 |
| 7 and 8 | Greensand, 4.04 grams. Sulfur, 1.35 grams; and manure, 1.35 grams. | 5.0 | 7650 |
| 9 and 10 | Greensand, 4.04 grams. Sulfur, 1.35 grams. Manure, 1.35 grams; and calcium carbonate, 5.0 grams. | 16.5 | 3400 |
| 13 and 14 | Compost, 7.57 grams†. | 0.7 | 11200 |
| 15 and 16 | Compost, 7.57 grams†; and calcium carbonate, 5.0 grams. | 20.9 | 2950 |
| 19 and 20 | Potassium sulfate, 0.5 grams. | 16.1 | 2050 |
| 21 and 22 | Potassium sulfate, 0.5 grams; and calcium carbonate, 5.0 grams. | 22.4 | 1000 |

* Average of 2 pots.
† Corrected for moisture and oxidation.

These results show that under favorable conditions on a soil low in potassium, an application of greensand alone increased the yield of

barley, while the treatment of lime and compost gave somewhat better results than lime and the same materials uncomposted. Whether this will be true after the first growing season will be shown by work in progress. An application of compost alone resulted in almost complete crop failure, but compost and lime gave practically the same results as potassium sulfate and lime. This indicates that when sufficient lime is used the potassium from the compost is practically equal in value to potassium from potassium sulfate. An interesting phase of this work is the acidity developed by the treatment with compost.

The pot culture tests show that when lime is not applied with the compost the yield of barley is seriously reduced. Observations, made while titrating to determine the water-soluble acidity of the compost, indicate the presence of large amounts of soluble iron and aluminium. From these indications it appears that this reduction in yield is due to an excess of soluble iron and aluminium salts which, upon the addition of lime, are converted into a form not injurious to barley. This is in agreement with the work of Hartwell and Pember[1]; Mirasol[2]; Ruprecht and Morse[3]; and Abbott, Conner, and Smalley[4].

As compared with the acidity of the soil treated with greensand alone, acidity determinations showed that in a single growing season the soil acidity resulting from the application of flowers of sulfur was equivalent to more than three times the theoretical amount of sulfuric acid that could be formed by complete oxidation of the sulfur. This is significant and worth keeping in mind when recommending applications of sulfur as a plant food, or for the purpose of combating the potato-scab disease.

The pot culture tests and chemical analysis of the compost show that the availability of the potassium in the compost is not decreased to an appreciable extent by the addition of lime, either before or after applying the material to the soil.

How may this information be of benefit to the average farmer? Since considerable labor is involved in making composts, it is probable that he will prefer to make direct additions of sulfur to soils well supplied with organic matter and total potassium. In this manner, through the process of sulfofication, it may be possible to obtain sufficient available potassium to meet the requirements of the usual field crops.

## SUMMARY.

(1) In the growth of barley the potassium contained in greensand-sulfur-manure compost was practically equal in availability to an equivalent amount supplied in the form of potassium sulfate.

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[1] *Soil Science*, 1918, 6: 259.
[2] *Ibid.*, 1920, 10: 175.
[3] Mass. Agr. Expt. Sta. Bull. 176: (1917).
[4] Purdue Univ. Agr. Expt. Sta. Bull. 170: (1913).

(2) An application of lime to the compost, or to the soil treated with the composted material, does not decrease the availability of the potassium, but is essential to secure increased yields.

(3) Greensand-sulfur-manure compost, when applied to a soil of low potassium content and deficient in organic matter, gave better yields at the end of the first growing season than an application of the same materials uncomposted.

A paper on "Some Results of the Determination of Potash by the Lindo-Gladding Method Using Alcohol of Various Strengths in the Presence of Sodium Salts" was presented by R. D. Caldwell and H. C. Moore of the Armour Fertilizer Works, Atlanta, Ga.

## REPORT ON INORGANIC PLANT CONSTITUENTS[1].

By J. H. MITCHELL (Clemson Agricultural College, Clemson College, S. C. ), *Referee.*

The work was divided into two parts. W. L. Latshaw was asked to develop a method for the determination of total phosphorus and, if possible, a better method for the determination of sulfur in the seeds of plants. A. J. Patten, the other associate referee, was asked to continue the work reported in 1919[2] on calcium and magnesium in the ash of seeds.

### RECOMMENDATIONS.

It is recommended—

(1) That further work be done on calcium and magnesium in the ash of seeds, as recommended in 1919[3].

(2) That some cooperative work be done on the colorimetric method for the determination of manganese.

(3) That a method be devised for the determination of iron and aluminium in the filtrate from magnesium, as recommended in 1919[3].

## REPORT ON SULFUR AND PHOSPHORUS IN THE SEEDS OF PLANTS[1].

By W. L. LATSHAW (Agricultural Experiment Station, Manhattan, Kans.), *Associate Referee.*

The purpose of the work was twofold:

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[1] Presented by R. N. Brackett.
[2] *J. Assoc. Official Agr. Chemists*, 1921, 4: 391.
[3] *Ibid.*, 395.

First, to develop a method for the determination of total phosphorus and, if possible, a better method for the determination of total sulfur in the seeds of plants.

Second, to make the determination from the same or aliquot portion of the sample.

The following material was used for analysis: soy bean meal; cottonseed meal; and mustard seed meal.

## RESULTS OF INVESTIGATION.

### MAGNESIUM NITRATE SOLUTION[1].

Twelve different concentrations of this solution were tried, each of the samples being used for the fusions. Small amounts of sodium carbonate were used with the magnesium nitrate to retard the oxidation. Magnesium nitrate can not be recommended as an oxidizing agent for the samples tried for the following reasons: (a) Oxidation is too vigorous and causes mechanical loss; (b) oxidation is incomplete unless a large amount of reagent is used, in which case the mechanical loss is proportionally increased.

### CALCIUM NITRATE SOLUTION.

Results with this reagent were similar to those with magnesium nitrate.

### FUSION MIXTURE.

*Sodium carbonate, 210 grams.*
*Potassium carbonate, 275 grams.*
*Potassium nitrate, 150 grams.*

Fusions were made with the above mixture in proportions of one of sample to ten of mixture. The oxidation was incomplete. The fusion mixture was tried again with the oxidizing reagent in the mixture increased to 225 grams. The results from cottonseed and soy bean meal with this mixture were good. The oxidation was complete and the determinations uniform. With the mustard seed, however, the oxidation was rapid and violent, resulting in mechanical loss.

### OFFICIAL METHOD[2].

The results with this method were fairly good, except with the mustard seed meal in which case the reaction with the sodium peroxide was violent and uncontrollable.

---

[1] *Assoc. Official Agr. Chemists, Methods.* 2nd ed., 1920, 2.
[2] *Ibid.*, 20.

### FUSION MIXTURE.

*Sodium hydroxide and potassium nitrate.*
*Potassium hydroxide and potassium nitrate.*
These fusion mixtures were tried but proved to be unsatisfactory.

### NITRIC ACID AND BROMINE.

Samples were evaporated to dryness with nitric acid and bromine then mixed with sodium carbonate and a small amount of potassium nitrate and ignited. This, however, was not successful.

### PARR BOMB.

A Parr bomb, such as is used for the determination of sulfur in coal, was employed with success, using sodium peroxide as an oxidizing agent with a small amount of finely ground potassium chlorate as an accelerator. Further work along this line was prevented because the bomb available for use could not accommodate more than a 0.3-gram sample with safety. The making of a bomb of a capacity sufficient to take care of a 2-gram sample is under consideration and should, when properly developed, prove a successful means of securing the complete oxidation of the seeds of plants and offer a solution of the problem for determining the total phosphorus and sulfur.

No report on calcium and magnesium in the ash of seeds was made by the associate referee.

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## FIRST DAY.

# MONDAY—AFTERNOON SESSION.—Continued.

## DRUG SECTION.

## REPORT ON DRUGS.

By G. W. Hoover (U. S. Food and Drug Inspection Station, Transportation Building, Chicago, Ill.), *Referee.*

The referee has made a partial review of the literature and desires to present to the association methods of analysis for drugs that have been published, which, in the opinion of the referee, are desirable for study with a view to their adoption as tentative or official. In the selection of published methods, consideration has been given, in general, to two important phases:

(1) The relative importance of drugs for which no tentative or official method exists.

(2) The selection of such methods of analysis for important drugs as give most promise of being satisfactory.

Brief abstracts of articles are submitted which contain methods for the examination of acetylsalicylic acid, phenolphthalein, camphor and camphor preparations, mercurous chloride, mercuric chloride, mercuric iodide, papain and turpentine. Innumerable methods of analysis and tests for drugs have been published. Since the plan of presenting published methods of analysis for drugs for study is an innovation, it seems desirable to proceed gradually to avoid making the plan cumbersome and unwieldy.

With this in mind, only a limited number of methods have been selected for presentation at this meeting.

The following are abstracts of the methods referred to and references to the literature where the different articles have been published:

### ACETYLSALICYLIC ACID.

This association has no tentative or official method for the examination of acetylsalicylic acid. The prominence of this drug has commanded the attention of various pharmaceutical chemists and during the last few years several very interesting and excellent articles have been published upon the subject. An attempt has been made to select and review

those articles which are believed to be of most use to a referee studying the subject of acetylsalicylic acid.

H. L. Schulz, in collaboration with C. K. Glycart, A. W. Hanson, and A. E. Stevenson, in an article entitled, "Methods of Analysis of Acetylsalicylic Acid and Adulterants"[1], gives the results of the analysis of samples of acetylsalicylic acid found upon the market. Qualitative tests are outlined for acetylsalicylic acid, free salicylic acid, salol, acetanilid, acetphenetidin, sugars, starch, and salts. A quantitative method for acetylsalicylic acid is also outlined. In this method, the acidity of acetylsalicylic acid is determined by dissolving the sample in 95 per cent alcohol, and titrating directly with 0.1N potassium hydroxide. Total acids are determined by hydrolysis with an excess of 0.1N potassium hydroxide, and the excess of alkali titrated back with 0.1N acid. The amount of acetylsalicylic acid is calculated by multiplying the number of cc. of 0.1N alkali used for the second or hot titration by the factor 0.018. A method for the determination of total salicylates is also given, which is based upon precipitation with standard iodine solution, and calculation of the salicylates from the weight of the precipitate.

A. J. Jones published an article on "The Purity of Commercial Aspirin and Aspirin Tablets"[2]. The author determined the acid and ester value of the sample dissolved in alcohol by direct titration with standard alkali before and after hydrolysis. The bromine value was determined by hydrolyzing a weighed sample with standard alkali on a water bath. Potassium bromide bromate reagent and hydrochloric acid were added, followed by sodium iodide, and the liberated iodine titrated with 0.1N sodium thiosulfate. The method was checked on pure salicylic acid.

In testing for free salicylic acid, 1 per cent ferric ammonium sulfate was used. The author states that both acetylsalicylic acid and alcohol have some effect on the color produced.

Paul N. Leech made a careful study of the physical and chemical properties of pure acetylsalicylic acid[3], and discussed the results of the examination of samples of various manufacturers. Especial attention was given to the determination of the melting point of this chemical.

A. Nutter Smith[4] did some very extensive work on a method for the determination of the extent of decomposition, especially with regard to the liberation of acetic acid. The phases of the subject that were taken up by him were studied very carefully, and the following summary is given:

(1) A new and accurate method of estimating free acetic acid in acetylsalicylic acid is described.

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[1] *J. Am. Pharm. Assoc.*, 1918, 7: 33.
[2] *Am. J. Pharm.*, 1919, 91: 461.
[3] *J. Ind. Eng. Chem.*, 1918, 10: 288; Rept. Chem. Lab. Am. Med. Assoc., 1919, 12: 62.
[4] *Pharm. J.*, 1920, 105: (4th ser., 51:), 90.

(2) It has been found that in the majority of instances free salicylic and acetic acids balance substantially; when acetic acid is in excess it is probably due to retention of acid due to incomplete purification, and when deficient it intimates that acetic acid has volatilized.

(3) The B. P. (British Pharmacopœia) coloration test for free salicylic acid is not very sensitive, and can easily be masked in a manner not so easily detected.

(4) Salts of acetylsalicylic acid appear to hydrolyze into free salicylic acid and an acetate of the base, and it is certain that they possess no advantage whatever over the acid as found on the market today, containing free salicylic acid in amount sufficient to produce the gastric disturbance usually attributed to the salicylates, the alleged objection which caused the latter to be superseded by the acetyl ester. It is not improbable that any therapeutic virtue or favor which the salts may possess over the acid is owing to the fixation of the acid on hydrolysis.

(5) Standards of 0.1 per cent free salicylic and 0.05 per cent free acetic acid are suggested for the drug, and double these amounts for tablets. If these limits are exceeded the controlling analyst would be justified in "failing" the sample, especially if recently prepared. It is well known that aspirin tablets on keeping tend to show an increase in the amounts of free acids present.

## METHODS FOR THE DETERMINATION OF PHENOLPHTHALEIN.

A. Mirkin published "A New Method for the Determination of Phenolphthalein"[1], which is based on the principle that phenolphthalein refluxed with hydroxylamine in an alkaline alcoholic solution is converted into its oxime. The method is similar to that used in the determination of camphor in which the excess of hydroxylamine is titrated. Complete details of the method and the necessary calculations are described. Mirkin states that accurate results have been obtained by his method.

Samuel Palkin published an article on "The Behavior of Phenolphthalein with Iodine and a Method for the Determination of Phenolphthalein"[2]. Palkin's brief description of this method follows:

A method has been devised for the accurate quantitative determination of phenolphthalein with a special adaptation to its medicinal preparations. This method is based on the quantitative yield of tetraiodophenolphthalein, when a solution containing phenolphthalein and iodine is alternately made alkaline to complete solution, and acid to complete precipitation, at a low temperature. The tetraiodophenolphthalein is determined by extraction with acetone-chloroform mixture, applying the usual method of immiscible solvents.

Palkin submits the results of analysis obtained by this method.

## METHODS FOR THE ASSAY OF CAMPHOR.

Several methods have been devised for the examination of camphor and preparations of camphor. Apparently, no single method has been given particular preference by pharmaceutical chemists. It seems de-

[1] *Am. J. Pharm.*, 1914, 86: 307.
[2] *J. Ind. Eng. Chem.*, 1920, 12: 766.

sirable to study the methods that have given most promise by workers in the pharmaceutical field.

H. C. Fuller outlined a procedure for "The Determination of Camphor"[1] which was based on Walther's carvone estimation and previous work of Nelson.

E. K. Nelson described a method for the determination of camphor[2] which depends upon the principle that camphor forms a well-defined oxime. The steps in the procedure are as follows:

A measured volume of an alcoholic solution of camphor, to which sodium bicarbonate is added, is refluxed with a standard solution of hydroxylamine. Hydrochloric acid is added to form hydroxylamine hydrochloride. Methyl orange is added and the mineral acid neutralized with normal alkali. Then phenolphthalein is added and the hydroxylamine hydrochloride titrated with 0.1N alkali. A blank is conducted and the difference in titrations represents the hydroxylamine converted into camphor oxime.

## POLARISCOPIC METHODS.

A. T. Collins published "A Method for Assaying Spirits of Camphor"[2], which directs that polarization be made in a 200 mm. tube, correcting for temperature (above or below 20°C.). He states that the rotation of different camphors varies. However, in the preparation of a control by evaporating and subliming a separate portion of the same sample, errors are eliminated. The presence of sugar is also accounted for in the non-volatile residue from sublimation. The method is claimed to be accurate.

Edwin Dowzard outlined a method for "The Determination of Camphor in Tablets and Pills"[3], which involves distillation with steam in a special apparatus to prevent blocking of camphor in the condenser tube. The apparatus is shown by cut. Results are calculated by polarizing the camphor, extracted with the aid of benzol, and comparing with a control. Accurate results are claimed by the author.

A number of years ago E. K. Nelson outlined a method for the determination of camphor in pills and tablets, which was never published. The method involves steam distillation. Blocking in the condenser tube is prevented by admitting a few drops of chloroform in the steam generator. The camphor is extracted from the distillate with chloroform, made to definite volume and polarized. At the same time, a 10 per cent solution of camphor and chloroform is polarized, and from the data obtained the amount of camphor in the sample is calculated.

## METHODS FOR THE DETERMINATION OF CALOMEL IN TABLETS.

In 1914, L. F. Kebler published an article on "The Tablet Industry—Its Evolution and Present Status"[4]. This article contains methods for

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[1] U. S. Bur. Chem. Circ. 77: (1911).
*J. Ind. Eng. Chem.*, 1912, 4: 514.
*Ibid.*, 1914, 6: 489.
[4] *J. Am. Pharm. Assoc.*, 1914, 3: 1062.

the analysis of calomel in tablets. Three methods for the determination of calomel are outlined, and the following are some of the essential points of each method:

*Method A.*—A weighed sample is disintegrated with potassium iodide. To this mixture is added standardized iodine solution. The excess of iodine is titrated with thiosulfate. Formulas showing the reaction and factor for calculation are given.

*Method B.*—This is a method by difference. The tablets are distintegrated in water, filtered upon a Gooch, and dried. The calomel is then volatilized over a Bunsen flame, and the amount of mercuric chloride in the tablets is calculated from the difference in weighings before and after volatilization.

*Method C.*—This is an indirect method. The sample of calomel is treated with sodium hydroxide, thus forming insoluble compounds of mercury (chiefly mercuric oxide) and sodium chloride. The chlorine is titrated with silver nitrate solution in the usual way. Formulas showing the reaction and factors for calculations are given.

In 1916, D. K. Strickland, in his article on "Laboratory Notes on the Standardization of the Mercurials"[1], essentially repeated the work published by Kebler. Strickland reports that the method in which the calomel is volatilized and calculations made from weighings before and after volatilization gives the most satisfactory results. The other two methods appear to have given low results, according to Strickland's comments.

## METHODS FOR THE ASSAY OF MERCURIC CHLORIDE TABLETS.

R. M. Chapin, in his article on "The Assay of Mercuric Chloride Tablets"[2], found that the method proposed by Rupp, modified by Smith, also modified and adopted by the German Pharmacopœia, gave good results with solutions of pure mercuric chloride. However, it was not applicable to commercial tablets or mixtures of mercuric and ammonium chlorides. The method of Rupp involved: (1) Reduction to metallic mercury by formaldehyde in alkaline solution in the presence of potassium iodide; (2) a solution of the precipitated mercury in excess of standard iodine solution after acidification with acetic acid; (3) titration of excess of iodine with sodium thiosulfate.

The author modified the method in the following respects: (1) The volume of liquid was increased by adding 75 cc. of water. This was found necessary to prevent the interference of hexamethyleneamine compounds and to prevent abnormalities in the presence of ammonium chloride; (2) the amount of potassium iodide was increased considerably to avoid the formation of a mercuric ammonium compound; (3) the time allowed for the reduction to metallic mercury was extended; (4) provision was made for running a blank against the reagents, especially to determine iodine-consuming materials known to be in formaldehyde.

The work by D. K. Strickland upon mercurials[1] reports results ob-

[1] *J. Ind. Eng. Chem.*, 1916, 8: 253.
[2] *Am. J. Pharm.*, 1914, 86: 1.

tained by the unmodified Rupp method. In the work reported by Kebler[1] essentially the original Rupp method is outlined for the determination of mercuric chloride. Accurate results are claimed for the modified method as published by Chapin.

Strickland found the original Rupp method satisfactory for hypodermic tablets, compressed tablets and pills. It is assumed that these preparations did not contain such interfering substances as referred to by Chapin.

Kebler and Strickland both outline the usual gravimetric method of determining mercury by precipitation with hydrogen sulfide. Strickland comments that the mercuric sulfide method has a tendency to give high results.

## METHODS FOR THE ASSAY OF MERCURIC IODIDE.

A. W. Bender in an article on "The Determination of Mercuric Iodide in Tablets"[2] states that while assaying tablet triturates for mercuric iodide he found difficulty, due mainly to other ingredients, such as terra alba, potato starch, and gelatin. The difficulty was overcome and accurate results secured by (1) digesting the tablets in hydrochloric acid in the presence of potassium chlorate; (2) precipitating mercury as sulfide with hydrogen sulfide in ammoniacal solution. Obviously, if the method is accurate, the sample must be free from other metals that may be precipitated with hydrogen sulfide in ammoniacal solution. The author claims the method is accurate and results of analyses and factors for calculations are given.

Strickland[3] reports upon determinations of mercuric iodide as follows:

> The method described under V (A) (original Rupp method for mercuric chloride) was successfully used for the determination of mercuric biniodide. Before applying it to various products it was tried out upon known quantities in the presence of varied amounts of sugar of milk. It was found that the sugar did not affect the result. Other factors in the reaction were varied. Thus it was observed that the time which the solution stood after alkali and formaldehyde were added was important. The most important factor, however, is the alkalinity of the solution. Unless there is a considerable excess of alkali present the mercury is not precipitated quantitatively; 30 minutes is the time required.

## METHODS OF ASSAY OF PAPAIN.

F. W. Heyl, C. R. Caryl and J. F. Staley published an article on the "Standardization of Commercial Papain"[4]. They experimented with different solutions for the purpose of ascertaining the media which gave the most satisfactory digestion. The experimental work was also con-

[1] *J. Am. Pharm. Assoc.*, 1914, 3: 1062.
[2] *J. Ind. Eng. Chem.*, 1914, 6: 753.
[3] *Ibid.*, 1916, 8: 253.
[4] *Am. J. Pharm.*, 1914, 86: 542.

trolled so as to exclude other ferments, such as pepsin. The authors discussed the meaning of the term "Papain" and reviewed salient points in methods of Graber, North, Rippetoe, Adams and Shelly. The following is a summary given by the authors:

1. In these digestions with pawpaw juice it has again been shown that the digestion proceeds rapidly at 80° to 100°C. This characteristic property can be utilized for the standardization of commercial papain samples.

2. Under the conditions outlined above, dried pawpaw juice should be capable of dissolving at 80° to 100°C. not less than 40 per cent of the egg-albumin taken.

3. No samples of "papain" were found on the market which had a higher digestive activity than the samples of dried pawpaw latex under the conditions employed.

4. Since the use of the term "papain" has given rise to the conditions pointed out in this paper, we are inclined to the view that papain products ought to be marketed as "dried pawpaw juice", and that only a lower limit of digestive strength should be stated in defining a standard for it. A definition proposed upon this basis might be stated as follows: Dried pawpaw juice is the dried albuminous exudate of the fruit of *Carica Papaya* L. (Fam. Papayaceæ), free from starch, sugars, and diluents, and contains a proteolytic enzyme or enzymes. When assayed by the method above[1] it has the power of digesting at 80° to 100° C. not less than 40 per cent of the unaltered egg-white protein.

5. Of twenty-six samples studied, seven represented the undiluted dried latex, fifteen contained starch in amounts varying from 15 per cent to 58 per cent, while three were diluted with sugar and one with dextrin. Four samples showed a high digestive strength under conditions favorable for pepsin digestion. On the basis of the standard proposed above, twelve samples, or 46 per cent, have been diluted to such an extent that their digestive strength is below a very reasonable requirement.

V. K. Chesnut made a "Report on Papain" in 1916[2]. Chesnut's work was upon authentic samples of papaya latex. He used uncoagulated casein prepared by the Hammerstein method to measure the proteolytic activity of the papain samples. The extent of the cleavage was measured with the polariscope. The great advantage of this method is the definiteness of the hydrogen-ion concentration. It is important to have the hydrogen-ion concentration within certain narrow limits, as the activity of the papain is much less above or below the optimum concentration.

Digestion requires 30 minutes. The unchanged protein is precipitated and filtered off. The filtrate is polarized in a 200 mm. tube to an accuracy of 0.01° to 0.02°. The amount of rotation is the measure of activity of enzyme. The rotation is nearly parallel to the amount of protein dissolved when small amounts of latex are used, but is not parallel when large amounts are used. This shows that the papain enzyme splits up the protein in certain ways, which are not shown by the amount of unchanged protein left.

The author states "the method here given is a comparatively simple

[1] *Am. J. Pharm.*, 1914, 86: 545.
[2] *J. Assoc. Official Agr. Chemists*, 1920, 3: 387.

one, very well adapted for research and seems well adapted for the assay of papain, especially where adulteration with other enzymes is suspected''.

## METHOD FOR THE DETECTION OF MINERAL OILS IN TURPENTINE.

A tentative method for the detection of mineral oil in turpentine is included in the methods of analysis of this association[1]. This method is based upon polymerization with fuming sulfuric acid.

A. E. Paul published an article on ''Turpentine and Its Adulterants''[2] which contains a method for the detection of mineral oil, based upon partial polymerization with sulfuric acid, and decomposition of the residue with fuming nitric acid. The following is the author's comment relating thereto:

> The modification, or rather the combination, of old methods, employed by the writer with entire success, is comparatively easy, is safe and rapid, includes all the possible fractions of petroleum products, and gives almost quantitative results, except in the case of very light naphtha, which, to the writer's knowledge, is never employed for the purpose of adulteration. Even in this case as much as 50 per cent of the amount present is readily separated, and as little as 1 per cent of added ordinary mineral product may be determined with certainty.

Data are submitted in support of the author's claim for the reliability of the method.

## RECOMMENDATIONS.

It is recommended—

(1) That an associate referee be appointed to study the methods for the examination of acetylsalicylic acid, reported herein or that may be elsewhere available, for the purpose of selecting or developing the most satisfactory method or methods of analysis.

(2) That an associate referee be appointed to study the methods for the examination of phenolphthalein, reported herein or that may be elsewhere available, for the purpose of selecting or developing a satisfactory method or methods of analysis.

(3) That an associate referee be appointed to study the methods for the examination of camphor and camphor preparations, reported herein or that may be elsewhere available, for the purpose of selecting or developing a satisfactory method or methods of analysis.

(4) That an associate referee be appointed to study the methods for the examination of mercurous chloride, mercuric chloride and mercuric iodide, reported herein or that may be elsewhere available, for the purpose of selecting or developing satisfactory methods of analysis.

(5) That an associate referee be appointed to study the methods for

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[1] *Assoc. Official Agr. Chemists, Methods.* 2nd ed., 1920, 306.
[2] *J. Ind. Eng. Chem.*, 1909, 1: 27.

the detection of mineral oils in turpentine, herein outlined, and any method or methods that may be elsewhere available, for the purpose of selecting or developing additional methods for the detection of mineral oils in turpentine.

(6) That an associate referee be appointed to study the methods of examination of papain, reported herein or that may be elsewhere available, for the purpose of selecting or developing satisfactory methods of analysis.

The writer wishes to acknowledge the kind assistance and interest of A. E. Paul, C. K. Glycart, A. W. Hanson, J. H. Bornmann, H. O. Moraw, F. W. Heyl, and S. Palkin in the preparation of this report.

## REPORT ON ALKALOIDS.

By A. R. Bliss, Jr. (Emory University, School of Medicine, Emory University, Ga.), *Associate Referee.*

In harmony with recommendations made at the 1919 meeting[1], samples were sent to collaborators, with instructions for their analysis, but the work has not sufficiently progressed to warrant a detailed report at this time. It is therefore recommended that the recommendations made at the 1919 meeting be continued.

## REPORT ON ARSENICALS.

By W. O. Emery (Bureau of Chemistry, Washington, D. C.), *Associate Referee.*

Some progress can be noted in the study of methods available for the chemical evaluation of arsenicals of the salvarsan type. The experience of the associate referee on arsenicals with the Ewens[2] and Lehmann[3] methods (oxidation with sulfuric and permanganic acid respectively) indicates that both are equally effective for determining the actual arsenic content, provided the digestion with sulfuric acid (Ewens' method) is carried out very slowly, and all subsequent operations in strict accordance with the directions of the author. In point of time and simplicity of operation, however, the method of Lehmann unquestionably possesses considerable advantage over that of Ewens, and is therefore to be recommended in ordinary chemical examinations of organic arsenicals.

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[1] *J. Assoc. Official Agr. Chemists*, 1921, **4**: 416.
[2] *J. Chem. Soc. (Trans.)*, 1916, **109**, II: 1355.
[3] *Apoth. Ztg.*, 1912, **27**: 545.

## REPORT ON SYNTHETIC DRUGS.

By C. D. WRIGHT (Bureau of Chemistry, Washington, D. C.), *Associate Referee.*

### RECOMMENDATIONS.

It is recommended—

(1) That the method for the valuation of hexamethylenetetramine tablets[1] be adopted as tentative.

(2) That the method of W. O. Emery for the estimation of monobromated camphor in migraine tablets[2] be studied cooperatively; and that the E. O. Eaton method be studied further.

(3) That the method of S. Palkin for the determination of phenolphthalein[3] be studied cooperatively; and that further study be made of other methods.

## REPORT ON METHODS OF ANALYSIS OF MORPHINE, CODEINE, AND DIACETYLMORPHINE (HEROINE).

By C. K. GLYCART (U. S. Food and Drug Inspection Station, Transportation Building, Chicago, Ill.), *Associate Referee on Alkaloids of Opium.*

No specific methods for the estimation of morphine, codeine, and diacetylmorphine (heroine) in tablets have been adopted by this association. For a number of years, in drug inspection work, many samples of alkaloidal salts of morphine, codeine, and heroine have been submitted for analysis. Various methods for the analysis of these drugs have been tried. In the course of the work an attempt has been made to prepare specific details for their analysis. The results obtained by the methods that have been devised were found satisfactory. Nothing new is claimed for the methods, but they are essentially an adaptation of the work that has been published.

### MORPHINE.

The following are the reagents required, instructions for the preparation of sample, and details of the method:

#### REAGENTS.

(a) *Alkaline salt solution.*—Dissolve 30 grams of sodium hydroxide in water, make to 1 liter, add sodium chloride to saturation and filter.

(b) *Chloroform-alcohol solution.*—Mix 90 cc. of chloroform with 10 cc. of alcohol.

(c) *0.02N sulfuric acid solution.*

(d) *0.02N sodium hydroxide (carbonate free).*

(e) *Methyl red.*—Dissolve 0.2 gram of methyl red in 100 cc. of alcohol.

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[1] *J. Ind. Eng. Chem.*, 1918, **10**: 606.
[2] *Ibid.*, 1919, **11**: 756.
[3] *Ibid.*, 1920, **12**: 766.

### PREPARATION OF SAMPLE.

Weigh separately at least 20 tablets to ascertain the variation in weight. Weigh collectively all unbroken tablets and calculate the average weight per tablet.

Weigh and transfer directly to a small separatory funnel a number of tablets representing 2 but not more than 3 grains of the alkaloid. Record the number of tablets used for the sample. In case the tablets contain more than ¼ grain of alkaloid, powder 20 or more tablets, mix thoroughly and protect from moisture in a weighing bottle. For each determination transfer to a small separatory funnel a weight of powdered material (equal to a multiple of the average weight per tablet), representing 2 and not more than 3 grains of the alkaloid.

### SEPARATION OF ALKALOIDS OTHER THAN MORPHINE.

Alkaloids other than morphine are extracted by chloroform, while morphine remains in the fixed alkali solution. In general, this separation is unnecessary (proceed to morphine). When tablets are of unknown composition, or atropine and hyoscine are present, shake the alkaline salt solution with 10 cc. portions of washed chloroform (use ether for the separation of atropine). Transfer the clear solvent to a small beaker, and evaporate on a steam bath. If a residue is obtained, apply the usual tests.

### DETERMINATION.

Moisten the sample in a separatory funnel with 5 cc. of water. Shake gently to disintegrate the tablets, then dissolve completely by adding 10 cc. of the alkaline salt solution.

To the alkaline salt solution, add a small piece of litmus paper, then concentrated hydrochloric acid, drop by drop, until neutral. Add 10 drops in excess. Then add 5 cc. of alcohol, carefully neutralize with ammonia, drop by drop, then add 5 drops in excess. (The addition of acid, ammonia, and alcohol should be made within the limits as directed. Ammonium chloride, which is sparingly soluble in alcohol, tends to neutralize morphine when the residue is heated.) Invert the separatory funnel and open the stop cock to insure neutralization of residual acid. (A cloudy precipitate in the ammoniacal salt solution does not interfere with complete extraction. It is necessary to keep the volume small in order to exhaust the alkaloid in the least number of extractions.) Immediately extract, at least six times, with chloroform containing 10% alcohol, using 30, 20, 20, 10, 10, 5 cc., or until the alkaloid is completely removed. (Test for the total removal of the alkaloid after the sixth extraction by adding 10 cc. of chloroform-alcohol solvent. Extract and evaporate in a separate beaker. Dissolve the residue in a few drops of neutral alcohol and add a drop of methyl red. Dilute with 20 cc. of water, carbonate free. A yellow color indicates incomplete extraction. Titrate as above and add value to total.) Combine the chloroform-alcohol extractions in a second separatory funnel into the stem of which is inserted a pledget of cotton wet with chloroform. Wash the combined extractions with 1 cc. of water. When clear, filter into a small beaker. Extract the wash water twice with small portions of chloroform-alcohol solvent. Evaporate on a water bath, using an electric fan to prevent decrepitation of the residue. Remove immediately when dry. Dissolve the residue with 2–3 cc. of neutral alcohol (water bath). Add 2–3 drops of methyl red indicator, then add from a buret 5–10 cc. excess of 0.02 N sulfuric acid, taking note of total amount used. Cover the beaker and heat to dissolve completely the residue adhering to the upper part of the beaker. Dilute with 50 cc. of cold, previously boiled water. Titrate back with 0.02 N alkali. The water and alkali should be sufficiently free from carbonates to insure a sharp end point with methyl red. The difference in the number of cc. of 0.02 N sulfuric acid added and the 0.02 N alkali required represents the amount of alkaloid.

1 cc. of 0.02 N acid = 7.52 mg. morphine hydrochloride + $3H_2O$ (U. S. P.), or
7.59 mg. morphine sulfate + $5H_2O$ (U. S. P.)

ALTERNATE METHOD FOR TITRATION OF ALKALOID.

If a sharp end point is not obtained with 0.02 N alkali and methyl red, the titration may be conducted as follows:

To the alkaloidal residue add 2–3 cc. of neutral alcohol, cover the beaker with a watch glass and heat on a steam bath until the residue adhering to the upper part of the beaker is completely dissolved. Add 2 drops of methyl red indicator and without dilution with water titrate carefully with 0.02 N sulfuric acid to a faint pink, avoiding an excess. Cover the beaker and digest on a steam bath until particles are completely dissolved. If more than 2 cc. of alcohol were added, evaporate the excess. Cool and dilute with 50 cc. of boiled water. (The solution should now be yellow.) Finish the titration with 0.02 N acid until a faint red.

COMMENT.

The method has been found to be rapid (1 hour), the alkaline salt solution facilitating the rejection of the alkaloid, and to yield accurate results. Further, provision is made for the separation of alkaloids, other than morphine.

TABLE 1.

*Typical results of analysis of morphine in commercial tablets.*

| PRODUCT | AMOUNT DECLARED | AMOUNT FOUND | SHORTAGE PER TABLET | SHORTAGE |
|---|---|---|---|---|
| | *grain* | *mg.* | *mg.* | *per cent* |
| Morphine sulfate hypodermic tablet | ¼ | 15.62 | 0.58 | 3.57 |
| Morphine sulfate tablet triturate | ¼ | 15.56<br>15.50 | 0.64<br>0.70 | 3.95<br>4.31 |
| Morphine sulfate compressed tablet | ¼ | 13.56<br>13.79 | 2.64<br>2.41 | 16.29<br>14.88 |
| Morphine sulfate tablet triturate | ¼ | 18.33<br>18.46 | 2.13*<br>2.26* | 13.14*<br>13.95* |
| Morphine sulfate tablet triturate | ¼ | 13.26 | 2.94 | 18.14 |
| Morphine sulfate hypodermic tablet | ¼ | 15.05<br>15.00 | 1.15<br>1.20 | 7.09<br>7.40 |

* Excess.

A morphine alkaloid (U. S. P.) was titrated direct to ascertain its purity which was 99.26 per cent. Three 100 mg. samples were prepared from the control and the following amounts were recovered: 98.94; 98.94; 98.85 mg., respectively. The last-mentioned analysis was made by H. O. Moraw.

CODEINE.

PREPARATION OF SAMPLE.

Proceed as directed under morphine, page 151.

DETERMINATION.

Dissolve the sample in a separatory funnel in the minimum amount of water (5 cc.) acidified with 2 drops of hydrochloric acid. (It is necessary to keep the volume small in order to exhaust the alkaloid with the least number of extractions.) Add solid sodium bicarbonate until slightly alkaline to litmus paper, extract five times with chloroform,

using about 30 cc. each time. (If morphine is present, reserve the alkaline solution for later use and proceed as directed below.)

Combine the chloroform extractions in a second separatory funnel into the stem of which is inserted a pledget of cotton wet with chloroform. Wash the combined extractions with 1 cc. of water containing 1 drop of ammonia, then proceed as directed under morphine, page 151, beginning with "Evaporate on a water bath".

1 cc. of 0.02 N sulfuric acid = 7.87 mg. of codeine sulfate + 5 $H_2O$ or 8.67 mg. of codeine phosphate + 2 $H_2O$ (U. S. P.).

SEPARATION OF MORPHINE.

Morphine and its derivatives are not dispensed together in the same tablet.

Codeine, which is administered in comparatively large doses, may contain small amounts of morphine as an impurity.

The separation is made as follows:

After complete extraction of codeine with chloroform, carefully neutralize the alkaline solution in the separatory funnel by adding concentrated hydrochloric acid slowly, drop by drop, then 10 drops in excess. Neutralize with ammonia, finally add 4 drops in excess. Add 5 cc. of alcohol. Extract with chloroform containing 10% alcohol. (See morphine.)

COMMENT.

Test for complete extraction of the alkaloid by evaporating a sixth extraction in a separate beaker. Dissolve the residue in a few drops of neutral alcohol. Add 1 drop of methyl red. Dilute with 20 cc. of water (carbonate free). A yellow color indicates incomplete extraction. Titrate as above and add value to total.

QUALITATIVE TESTS FOR CODEINE AND MORPHINE.

| REAGENTS | CODEINE | MORPHINE |
|---|---|---|
| Concentrated nitric acid | Yellow | Red |
| Boiling ether | Soluble | Insoluble |
| Solution of fixed alkali | Insoluble | Soluble |
| 1 drop of ferric chloride solution in dilute potassium ferricyanide solution | Gradually turning green | Blue |
| Microchemical test: | | |
| Add 1 drop of 0.1 N iodine to alkaloid, dissolved in 1 drop of 0.1 N hydrochloric acid on slide | | Characteristic crystals, under low power lens |

TABLE 2.

*Typical results of analysis of codeine in commercial tablets.*

| PRODUCT | DECLARED | FOUND | SHORTAGE PER TABLET | SHORTAGE |
|---|---|---|---|---|
| | *gram* | *mg.* | *mg.* | *per cent* |
| Codeine sulfate tablet triturates | ½ | 30.85 | 1.55 | 4.78 |
| | | 31.16 | 1.24 | 3.83 |
| Codeine sulfate tablet triturates | ¼ | 14.95 | 1.25 | 7.71 |
| Codeine sulfate hypodermic tablets | ¼ | 15.06 | 1.14 | 7.04 |
| | | 15.03 | 1.17 | 7.22 |
| | | 16.37 | 0.17* | 1.05* |
| Codeine phosphate hypodermic tablets | ¼ | 16.10 | 0.10* | 0.61* |

*Excess.

## HEROINE (DIACETYLMORPHINE).

### PREPARATION OF SAMPLE.

Proceed as directed under morphine, page 151, through sentence reading: "Record the number of tablets used for the sample".

### DETERMINATION.

Dissolve the sample in a separatory funnel in 5 cc. of water, containing 1 drop of acetic acid. Add 1 cc. of ammonia. Extract five times with 25 cc. portions of chloroform. Combine the chloroform extractions in a second separatory funnel, into the stem of which is inserted a pledget of cotton wet with chloroform. Wash the combined extractions with 1 cc. of water. Then proceed as directed under morphine, page 151, beginning with "Evaporate on a water bath".

1 cc. of 0.02 N sulfuric acid = 8.48 mg. diacetylmorphine hydrochloride + $H_2O$ (U. S. P.).

### COMMENT.

Mineral acids and solutions containing fixed alkali, readily decompose diacetylmorphine to morphine and acetic acid. The above method precludes the possibility of decomposition in the process of analysis.

### SEPARATION OF MORPHINE.

Diacetylmorphine may decompose with age, turning tablets to varying shades of gray. For purposes of estimation of decomposition, the morphine is extracted by treating the ammoniacal solution remaining in the separatory funnel with chloroform containing 10% alcohol. (See morphine.)

*Qualitative test.*—Heat the diacetylmorphine residue with concentrated sulfuric acid and a small quantity of alcohol. Ethyl acetate is formed, which is recognized by its odor.

*Microchemical test*[1].—Crystals with platinic chloride. Transfer a fragment of the alkaloid to a perfectly clean glass slide and drop upon it, from a buret, 1 drop of 0.1N hydrochloric acid. When completely dissolved, add a drop of 10% platinic chloride solution and, without stirring or otherwise disturbing the mixture, examine under low power of microscope. The precipitate as first formed is amorphous, but within a minute or so, in the case of pure heroine (10–15 minutes in the case of mixtures), clusters of needles form around a nucleus. In a short time the needles begin to fly off and continue until the whole cluster is disintegrated. This test is infallible for this alkaloid.

TABLE 3.

*Typical results of analysis of (diacetylmorphine) heroine in hypodermic tablets.*

| PRODUCT | DECLARED | FOUND | SHORTAGE PER TABLET | SHORTAGE |
|---|---|---|---|---|
| | *grain* | *mg.* | *mg.* | *per cent* |
| Diacetylmorphine hydrochloride | $\frac{1}{12}$ | 5.40 | 0.00 | 0.00 |
| Diacetylmorphine hydrochloride | $\frac{1}{12}$ | 5.20 | 0.20 | 3.70 |
| Diacetylmorphine hydrochloride | $\frac{1}{12}$ | 5.12<br>5.19 | 0.28<br>0.21 | 5.18<br>3.90 |
| Diacetylmorphine hydrochloride | $\frac{1}{12}$ | 4.45<br>4.70 | 0.95<br>0.70 | 17.60<br>13.00 |

[1] *J. Ind. Eng. Chem.*, 1912, 4: 508.

RECOMMENDATION.

It is recommended that the methods herein submitted for the assay of morphine, codeine and heroine be studied during the coming year with a view to their final adoption by the association.

## REPORT ON MEDICINAL PLANTS.

By ARNO VIEHOEVER (Bureau of Chemistry, Washington, D. C.), *Associate Referee.*

The report is divided into four parts:

I. Detection of molds in drugs, foods and spices, by means of the chitin test.

II. Identification and differentiation of plants and plant products by means of the pollen grains.

III. Value of weights of unit volumes or the specific weight of crude drugs and spices.

IV. Important adulterants and substitutes of crude drugs and spices.

### PART I.

The importance of the detection of the presence of mold in drugs, foods and spices is very evident. Where the growth of mold is very conspicuous, covering to a greater or less degree the particular product, analytical proof is usually superfluous. A cultural and microscopic study of the mold in such instances can readily be made. Where, however, the material is not conspicuously infested on the outside, or where it is processed by powdering or otherwise, thus materially changing the appearance of the product, the problem of detecting and identifying the mold becomes far more difficult of solution. Cases are not rare in plant or animal pathology where it is desired to establish the extent of infestation or mold growth in the tissues. Especially serious is the problem where molds, poisonous even in very small amounts, are suspected in food products, such as ergot in flour, or ergotized spices, such as ergotized caraway and cumin.

Mainly to facilitate the task of the analyst, in such cases, a method is described, based on the presence of a specific chemical substance, chitin, in the cell wall of molds and other fungi cells. This substance, by a special treatment, is transformed in such a manner that it can be specifically stained. The subject has been discussed by the writer at some length[1].

The following method has been suggested for the treatment and staining of the mold-infested material:

[1] *J. Am. Pharm. Assoc.*, 1917, 6: 518.

### METHOD.

Heat the material containing the mold almost to boiling with 40–50% potassium hydroxide or sodium hydroxide for 40–60 minutes. The heating may be done conveniently on an electric plate, in a flask, the opening of which is covered with a funnel.

After centrifugalizing, if necessary, decant the excess of potassium hydroxide. If practicable, press out the material with a glass rod to remove as much as possible of the potassium hydroxide. Then wash with alcohol or glycerol (about 50%) depending on the nature of the product.

### STAINING OF CHITOSAN.

After removing the last traces of potassium hydroxide with dilute alcohol or glycerol (about 25%), and possibly neutralizing at the end with 1% sulfuric acid, treat the material with a solution of iodine potassium iodide (2 parts of iodine, 1 of potassium iodide, and 200 cc. of water). Then replace the excess of iodine with dilute sulfuric acid, preferably 1%. In the presence of chitosan a distinct red to violet color is detected.

### SPECIAL REMARKS.

If the treatment with potassium hydroxide is not unduly extended, the plant tissue is not very much destroyed and the stain brings out the mold mycelium very distinctly.

In case the color should not be distinct or even be covered with another color in the hyphae, the untreated material may, according to van Wisselingh[1] and Wester[2], first be heated with glycerol to 300°C. and then treated with potassium hydroxide.

If the material contains large amounts of starch, which may give a color somewhat similar to that of chitosan, the starch can be hydrolyzed with freshly prepared diastase from malt or with taka-diastase or can be differentiated from the mold with the polarization microscope through its ability to refract the light.

Since alkali carbonates do not seem to effect the transformation of the cell substance, chitin, as well as hydroxides, it is essential that the solution of alkali hydroxides used should not contain considerable amounts of carbonates. After treatment with potassium hydroxide it is often advisable to make the test with part of the material transferred to a watch glass or object slide. It is important that the free potassium hydroxide be removed completely since otherwise the iodine solution will be discolored and prevent the stain from developing.

The material must be actually stained with iodine solution before replacing the iodine with dilute sulfuric acid. For purposes of preservation, the preparation is best kept in dilute glycerol (1 to 1). The stain becomes gradually weaker and disappears after about 24–48 hours.

Copies of the method and a sample of ergotized caraway, as well as moldy areca nuts, were submitted to a number of collaborators, with the request that the test be tried. The following replies were received:

*E. O. Eaton.*—The test appears, on limited application, to be of value. I obtained positive results on known molds treated as directed, as well as in samples submitted.

*G. L. Keenan.*—Several fragments of ergot were heated, etc., (according to method). A distinct violet-red color was obtained.

*Miss M. B. Church.*—I have tried the chitin test mainly on canned strawberries, rotten cherries, molded soy beans and occasional crude drugs. Attempts to use it on

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[1] *Jahr. wiss. Botanik*, 1898, 31: 619.
[2] Studien über das Chitin. *Inaugural Dissertation*, Bern. (1909).

lumps of moldy flour were not satisfactory, because of the awkwardness natural to handling starchy stuff with a hydroxide in such technique. We have never been interested in centrifugalizing minute amounts of material treated with the chitin test and examining minute quantities of residue for fungal cellulose, because occasional mold or bacteria is regarded as of no importance for our work. Therefore I can not discuss the fineness of the method to any extent. I have always been able ultimately to make the chitin test work. I have been successful in training my eye to recognize to my positive satisfaction the shade of pinkish purple which means fungal cellulose. I have never seen any other material staining this particular shade.

*C. W. Ballard.*—I am very much interested in the question of molds in foodstuffs and have used the method described in your article. I find that the results are good and certain. In one investigation it was necessary to identify small quantities of mold in canned pumpkin. You know how closely broken mold filaments resemble broken cellulose walls. Nevertheless, the method was used. In this instance, the parenchyma cell walls were stained blue and one had to depend upon structural characters as well as staining properties. I have also used the method in working with condensed milk containing mold buttons. In this instance it was necessary to work with dilute solutions and centrifugalize.

*E. N. Gathercoal.*—I made the test on the two samples that you sent me, and found it to work very well, indeed. I also tried it on a sample of moldy bread, but found that the detection of the mold hyphae was difficult because the starch was not completely destroyed. The test would appear to be a very satisfactory one for the detection of fungi in powdered drugs where the fungal threads would be difficult to locate among the other tissues without this special treatment. However, in material such as bread, preserved fruits, etc., the usual microscopic mounts would be much quicker, and perhaps as satisfactory for the detection of the mold threads.

*J. F. Clevenger.*—Various products in the course of routine work, such as moldy coffee, moldy oats, and certain spices or drugs infested by mold, were treated according to the method suggested in the chitin test. The stain could always be obtained, although it seems necessary to follow the procedure suggested quite carefully, and advisable to prolong the treatment of boiling, etc., in cases where the mold hyphae are only partly and indistinctly stained.

## PART II.

With regard to the use of pollen grains in the differentiation of drugs and other plant products, the main thought is to utilize the means of differentiation readily accessible in nature as in herbariums, and yet evidently very much neglected by botanists and analysts in the identification of plants and plant products. From a study of the data given in literature, as well as from the limited data secured in laboratory analyses, it is believed that the pollen grain has a specific and constant structure. It is true, as far as morphological classes are concerned, mainly flowers and herbs collected in the flowering state will be suitable material for testing.

In the microscopical examination of honey, pollen grains are used to ascertain the origin of the honey. It is hoped that the use of pollen grains can be extended. Many plants of economic or medicinal value are collected during the flowering period. Most herbarium specimens are collected during that time, inasmuch as the taxonomists base the

identification, whenever possible, on floral characteristics. By the use of the outline and size of the pollen grains and the morphological structure of the exine, and correlating the results with other means of identification and differentiation of varieties, genera, and families, very useful data will be at hand to establish the identity of the product.

Perhaps it may not always be desirable or possible to depend upon this pollen character alone, but the accessibility of the pollen, the uniformity in structure, the specific character, and the fact that it is not subject to decomposition, will speak strongly in favor of its adoption for taxonomical purposes. It is believed that a most desirable asset to a collection of drugs and other plant products would be a collection of pollen grains associated with the individual drug samples, which would, no doubt, in many instances, make the authenticity of the material unquestionable.

The following method was tentatively suggested:

METHOD.

Place the material containing floral structures, especially pollen grains, on a microscopical slide, mix with dilute glycerol (1 to 1), or a mixture of equal amounts of chloral hydrate solution (8 to 5) and glycerol. After covering with a cover glass, examine under the microscope. The magnification to be used varies with the size of the pollen grains, but usually a magnification varying from 400–600 will be found satisfactory. Examine the pollen grains, which are recognized by their characteristic structure, as to size, outline, and morphological structure of the exine, the outer pollen grain wall. Also determine the number of openings for the pollen tube. In some instances treatment with concentrated sulfuric acid may be found to give a characteristic reaction, such as with pollen grains of Mallow (*Malva sylvestris* L.), which are colored distinctly red upon the addition of sulfuric acid.

Pollen grains in liquid suspension, such as honey, are readily found in the sediment, if necessary, after dilution and centrifugalizing of the liquid, or, preferably, after settling in sediment tubes or apparatus.

A number of collaborators were approached with the request to relate the extent of their experience, if any, in the use of pollen grains as a diagnostic means for differentiation of drugs and other plant products.

*C. W. Ballard.*—This procedure is satisfactory in working with herbs and aboveground portions but, in my opinion, is of little use in working with other parts of the plant.

*E. N. Gathercoal.*—We have had little experience regarding the use of pollen grains as a diagnostic means for differentiation of drugs and other plant products. We have met with pine pollen as a substitute for lycopodium and some years ago we found a good deal of pollen in a sample of powdered belladonna leaves. We decided that this was belladonna pollen and that the sample consisted mostly of flowering tops.

Albert Schneider[1] discusses the value of pollen grains in drug analysis and appears to be in favor of their use as diagnostic elements.

[1] Microanalysis of Powdered Vegetable Drugs. 2nd ed., 1921, 102.

The writer has collected a great number of data from literature and, with the aid of J. F. Clevenger, has established the characteristic pollen grain structures of several drugs, such as *Onopordon*, *Convallaria*, and *Matricaria* (chamomile).

## PART III.

The work on the value of weights of unit volumes of crude drugs and spices, discussed in previous reports[1], was continued.

C. J. Zufall submitted a large number of interesting data, which demonstrated the usefulness of the method. (See Table 1.) Inasmuch, however, as it was feared that the individual factor of filling the cylinder with material may vary considerably and cause unsatisfactory results, the work was continued along a line which appeared to eliminate this factor of chance. An apparatus devised by Kunz-Krause[2] in 1919 for the determination of the apparent or absolute specific weight of substances was used. It consisted of a flask not unlike a picnometer, the upper part ending in a narrow tube and fitting with a ground joint into a glass of suitable size. The volumes in the two flasks used were 62 and 142. Kerosene, made moisture free with anhydrous sodium sulfate, was used according to the suggestion of Kunz-Krause. A known amount (about 20 grams) of the vegetable material to be tested in whole or ground state, was filled into the lower part after about 20 cm. of kerosene had been previously introduced into the flask, using the larger one for the data reported.

The apparatus was put together, using a drop of water to effect a good joint of the ground faces, and kerosene filled in from a buret to the mark indicated on the neck of the flask. The difference in volume of kerosene needed to fill to the mark, representing the amount of liquid displaced by the added vegetable material, divided by the actual weight of the drug added, represents the desired result. Where drugs in a whole state are used this is identical with the apparent specific weight; where the finely ground material is used, it is identical with the absolute specific weight.

Data thus obtained are tabulated in Table 2.

[1] *J. Assoc. Official Agr. Chemists*, 1920, 4: 149; 1921, 4: 409.
[2] *Ber. pharm. Ges.*, 1919, 29: 147.

TABLE 1.

*Weight per unit volume of some crude drugs.*

(Analyst, C. J. Zufall.)

| DESCRIPTION OF SAMPLE | WEIGHT DIVIDED BY VOLUME | REMARKS |
|---|---|---|
| Anise seed | 0.377* | Average appearance. Rock fragments, 1%; stems, 0.5%. |
| Anise seed | 0.363* | Average appearance. Sand, 0.5%; stems and foreign matter. |
| Anise seed | 0.360* | 2% fine sifting of stems and anise seed fragments. |
| Anise seed | 0.376 | 1% sand. |
| Anise seed | 0.372 | Average appearance very clean. |
| Anise seed | 0.380 | Average appearance very clean. |
| Anise seed | 0.397 | Average appearance very clean. |
| Anise seed | 0.445 | N. Y. 79655. Exhausted seeds 65%, much dust. |
| Anise seed | 0.333 | Exhausted completely with steam in laboratory. |
| Anise seed | 0.382 | Contains 10% seeds exhausted in laboratory. Grade A, 90%. |
| Anise seed | 0.387 | Contains 20% seeds exhausted in laboratory. Grade A, 80%. |
| Anise seed | 0.370 | Contains 50% seeds exhausted in laboratory. Grade A, 50%. |
| Anise seed | 0.341 | Partially exhausted in laboratory. |
| Anise seed | 0.315* | Partially exhausted in laboratory. |
| Caraway seed | 0.478 | Dutch. Very clean. |
| Caraway seed | 0.477 | Dutch. Very clean. |
| Caraway seed | 0.496 | Dutch. Very clean. |
| Caraway seed | 0.457 | Dutch. Very clean. |
| Caraway seed | 0.466 | Dutch. Very clean. |
| Caraway seed | 0.470 | Dutch. Very clean. |
| Caraway seed | 0.498 | Dutch. Very clean. |
| Caraway seed | 0.469 | Dutch. Very clean. |
| Caraway seed | 0.499 | Dutch. Very clean. |
| Caraway seed | 0.503 | Dutch. Very clean. |
| Caraway seed | 0.504 | African. Pedicels, 1%, and stems, 1%. |
| Caraway seed | 0.501 | African. Very clean. |
| Coriander | 0.310 | No stems, soil or foreign matter. Normal appearance. |
| Coriander | 0.311 | No stems, soil or foreign matter. Normal appearance. |
| Coriander | 0.326 | No stems, soil or foreign matter. Normal appearance. |
| Coriander | 0.306 | No stems. Soil particles, 1%, same size as coriander. |
| Coriander | 0.304 | No stems. Soil, 1%. Shaking produced no change in volume. |
| Coriander | 0.294 | Soil, 0.5%. |
| Celery seed | 0.503* | No dust. Sand, 0.5%. |
| Celery seed | 0.476 | Sand, 1%. Very little dust. |
| Cubebs | 0.306 | Average appearance. Stems, 5%. No other foreign matter. Many small fruits. |
| Cubebs | 0.262* | Average appearance. Stems, 4%. |
| Cubebs | 0.190* | Fruits very small. Stems, 50% and ¼ to 1 inch in length. |
| Cumin seed | 0.305* | Average appearance. Small rocks, 0.5%. |
| Cumin seed | 0.324* | Average appearance. Small rocks, 1.5%. |
| Cumin seed | 0.364* | Average appearance. Very clean. |
| Cumin seed | 0.350* | Average appearance. Very clean. |
| Cumin seed | 0.360 | Average appearance. Very clean. |
| Cumin seed | 0.373 | Average appearance. Few small pebbles. |
| Fennel seed | 0.325* | Average appearance. Less than 1% sand. |
| Fennel seed | 0.276* | Average appearance. Large German. Very clean. |
| Fennel seed | 0.310 | Average appearance. Large German. Very clean. |
| Fennel seed | 0.296 | Average appearance. Large German. Very clean. |
| Fennel seed | 0.301 | Average appearance. Large German. Very clean. |
| Fennel seed | 0.301 | Average appearance. Large German. Very clean. |

TABLE 1.—Continued.

| DESCRIPTION OF SAMPLE | WEIGHT DIVIDED BY VOLUME | REMARKS |
|---|---|---|
| Fennel seed | 0.694 | Small French bitter fennel. Old, dark and moldy. |
| Fennel seed | 0.389 | Medium size. German. Very clean. |
| Fennel seed | 0.377 | Medium size. German. Very clean. |
| Fenugreek seed | 0.723 | Soil, 0.5%. No other foreign matter. |
| Juniper berries | 0.430 | Less than 1% brown fruits. |
| Juniper berries | 0.383 | Shrivelled fruits, 1%. |
| Lycopodium | 0.378 | No starch or foreign matter. Very dry. Ash, 1%. |
| Lycopodium | 0.363 | No starch or foreign matter. Very dry. Ash, 1.2%. |
| Lycopodium | 0.408 | No starch. Foreign vegetable matter, 0.3%. Ash, 1.77%. |
| Lycopodium | 0.340* | No starch or foreign vegetable matter. Ash, 11.6%. |
| Lycopodium | 0.400* | Starch, 20%. No foreign vegetable matter. Ash, 8%. |
| Marjoram | 0.108* | Average. No Coriaria. Very clean. |
| Marjoram | 0.109* | Average. No Coriaria. Very clean. |
| Marjoram | 0.096* | Average. No Coriaria. Very clean. |
| Marjoram | 0.125 | Average. No Coriaria. Very clean. |
| Marjoram | 0.106* | Average. No Coriaria. Very clean. |
| Marjoram | 0.136 | Average. No Coriaria. Very clean. |
| Mustard seed | 0.710 | Average. Very clean. English yellow. |
| Mustard seed | 0.735 | Average. Very clean. English yellow. |
| Mustard seed | 0.737 | Average. Very clean. English yellow. |
| Mustard seed | 0.755 | Average. Very clean. English yellow. |
| Mustard seed | 0.752 | Average. Very clean. English yellow. |
| Mustard seed | 0.744 | Average. Very clean. English yellow. |
| Mustard seed | 0.717 | Average. Very clean. English yellow. |
| Mustard seed | 0.707 | Average. Very clean. English yellow. |
| Mustard seed | 0.712 | Average. Very clean. English yellow. |
| Mustard seed | 0.712 | Average. Very clean. English yellow. |
| Mustard seed | 0.731 | Average. Very clean. English yellow. |
| Mustard seed | 0.717 | Average. Very clean. English yellow. |
| Mustard seed | 0.686 | Average. Very clean. English yellow. |
| Mustard seed | 0.688 | Average. Very clean. English yellow. |
| Mustard seed | 0.719 | Average. Very clean. English yellow. |
| Mustard seed | 0.723 | Average. Very clean. English yellow. |
| Mustard seed | 0.719 | Average. Very clean. English yellow. |
| Mustard seed | 0.713 | Average. Very clean. English yellow. |
| Mustard seed | 0.751 | Average. Very clean. English yellow. |
| Mustard seed | 0.719 | Slightly moldy. Foreign seeds, 4%. English yellow. |
| Mustard seed | 0.751 | Slightly moldy. Foreign seeds, 3%. English yellow. |
| Mustard seed | 0.738 | Danish yellow. Very clean. |
| Mustard seed | 0.678 | Chinese yellow. Very clean. |
| Mustard seed | 0.687 | Danish brown. Very clean. |
| Mustard seed | 0.682 | Danish brown. Very clean. |
| Mustard seed | 0.695 | Danish brown. Very clean. |
| Poppy seed | 0.636 | Blue. Very clean. |
| Thyme | 0.236 | Very clean. |
| Thyme | 0.199 | Very clean. |

* Drug not shaken down. All other results from shaking sample down as far as it would go. Nearly all samples consisted of 1000 cc.

TABLE 2.

*Specific gravity and other data* for crude drugs and spices.*

| PRODUCT | CONDITION | SPECIFIC GRAVITY | WEIGHT PER 500 cc. | ASH | | REMARKS |
|---|---|---|---|---|---|---|
| | | | | Total | Acid-insoluble | |
| | | | *grams* | *per cent* | *per cent* | |
| Pepper, Tellichery | Whole | 1.11 | 276 | .... | .... | Good grade |
| Pepper, Tellichery | Ground No. 60 | 1.33 | ... | 4.2 | 0.3 | Good grade |
| Pepper, Acheen | Whole | 1.00 | 202 | .... | .... | Poor quality |
| Pepper, Acheen | Ground No. 60 | 1.29 | ... | 5.6 | 1.4 | Poor quality |
| *Veratrum viride* | Fine powder | 1.53 | ... | 19.0 | 15.2 | |
| *Veratrum album* | Fine powder | 1.43 | ... | 4.9 | 2.0 | |
| *Veratrum album* | Coarse powder | 1.33 | ... | 6.2 | 3.1 | Roots only |
| Black mustard (*Brassica nigra*) | Whole seed | 1.16 | ... | .... | .... | |
| Rape, (*Brassica napus*) | Whole seed | 1.08 | ... | .... | .... | |
| Mustard, (*Brassica besseriana*) | Whole seed | 1.17 | ... | .... | .... | |
| Chinese colza (*Brassica campestris* var. *Chinoleifera* Viehoever) | Whole seed | 1.11 | ... | .... | .... | |
| White mustard (*Sinapis alba*) | Whole seed | 1.23 | ... | .... | .... | |

* Data secured by J. F. Clevenger and Ruth G. Capen.

The difference in weight observed suggests the usefulness of the method. The presence of ash in the sample of *Veratrum* is clearly indicated by the higher specific weight. Further work must, of course, be done.

## PART IV.

Samples of sage (*Salvia officinalis*) put up in small packages, proved to be Greek sage (*Salvia triloba* L.). Powdered capsicum proved to be a species other than *frutescens*, official in the U. S. Pharmacopœia. A sample labeled "Allspice" was identified as *Vitex agnus-castus* L.

Among the imported drugs found to be substituted should be mentioned cubebs, containing close to 75 per cent of the fruit of another *Piper* species, *Piper ribesioides* Valerian U. S. P. substituted by a Mexican species, as well as by an Ecuadorian species; Convallaria flowers (*Convallaria majalis*) for *Matricaria*; *China rubra* for cascara.

A very skilful fake saffron was offered for entry. It was proved to consist entirely of flowers resembling the common thistle and representing the species *Onopordon sibthorpianum* Boiss and Heldr. This was artificially colored with a red dye, Ponceau 3 R, and a yellow dye, tartrazine. It was weighted with a salt mixture of potassium nitrate, borax and also glycerol and evidently flavored with saffron oil.

## RECOMMENDATIONS.

It is recommended—

(1) That the method for the detection of molds in drugs, foods and spices, by means of the chitin test, be adopted as a tentative method.

(2) That the method for the use of pollen grains as a means of identification and differentiation of plants and plant products be further studied.

(3) That further work be done on the value of weights of unit volumes or the specific weight of crude drugs and spices.

(4) That further information be collected concerning adulterants and substitutes of crude drugs and spices.

No report on enzymes was made by the associate referee.

# METHOD FOR THE EXAMINATION OF PROCAINE (NOVOCAINE)[1].

By ALFRED W. HANSON (U. S. Food and Drug Inspection Station, Transportation Building, Chicago, Ill.).

In the examination of procaine it is well recognized that the base may be extracted from an ammoniacal solution with ether or chloroform and determined by titration in the usual manner employed for alkaloids.

In studying the chemical properties of procaine, it was found that it can be titrated directly with potassium bromide-bromate reagent after first hydrolyzing. Details of a method based upon this principle have been devised and the results of the examination of samples of procaine appear to be quite satisfactory.

In examination of this drug it seems advisable that qualitative tests be made for the purpose of identification.

### REAGENTS.

(a) *Mercuric potassium iodide (Mayer's reagent).*—Dissolve 1.3 grams of mercuric chloride in 60 cc. of water, add 5 grams of potassium iodide dissolved in 10 cc. of water and make to 100 cc.

(b) *Potassium permanganate solution.*—Dissolve 5 grams of potassium permanganate in water, and make to 100 cc.

### PHYSICAL AND CHEMICAL TESTS.

(1) Procaine melts at 153–155°C.

(2) Dissolve 0.1 gram of procaine in about 10 cc. of water. When this solution is treated with 2 cc. of the potassium permanganate solution, reduction occurs with evolution of gas having the odor of acetaldehyde (distinction from cocaine, which does not readily reduce potassium permanganate).

[1] Presented by G. W. Hoover.

(3) Dissolve about 5 mg. of the sample in 5 cc. of water, adding a few drops of mercuric potassium iodide (Mayer's reagent). In the case of procaine, a white precipitate is formed which dissolves if a few cc. of dilute sulfuric acid are added. (The precipitates with mercuric potassium iodide (Mayer's reagent) formed with stovaine and cocaine are not readily soluble in dilute sulfuric acid.)

(4) Dissolve about 0.1 gram of procaine in 5 cc. of water. Add 2 drops of dilute hydrochloric acid, 2 drops of 10% sodium nitrite solution, and mix with a solution of 0.2 gram of betanaphthol in 10 cc. of 10% sodium hydroxide solution. A scarlet red precipitate is formed.

(5) To a solution of about 0.1 gram of procaine in 5 cc. of water, add 3 drops of dilute sulfuric acid, and mix with 5 drops of 0.1 N potassium permanganate. The violet color of the latter disappears immediately (distinction from cocaine).

(6) Dissolve about 0.1 gram of procaine in 1 cc. of sulfuric acid. The solution is colorless (organic impurities).

### REAGENTS.

(a) *0.1N sodium hydroxide (for hydrolyzing upon steam bath).*

(b) *0.1N sodium thiosulfate.*

(c) *Potassium iodide solution.*—Dissolve 20 grams of potassium iodide in water and make to 100 cc.

(d) *Potassium bromide-bromate solution.*—Dissolve 3 grams of potassium bromate and 15 grams of potassium bromide in water. Make to 1 liter (this should be standardized against 0.1 N sodium thiosulfate).

(e) *Starch indicator.*—Mix about 0.5 gram of finely powdered starch with cold water to a thin paste, pour into about 100 cc. of boiling water, stirring constantly, and discontinue heating immediately after the paste is added.

### DETERMINATION.

Dissolve 0.1 gram of the sample in 5 cc. of water in a 50 cc. beaker. Add 25 cc. of 0.1 N sodium hydroxide and heat upon a steam bath for 25 minutes. Cool, transfer the solution to a 500 cc. Erlenmeyer flask, having a tightly fitting ground glass stopper. Add 50 cc. of accurately measured standardized potassium bromide-bromate solution. Dilute with water to 250 cc. Add 10 cc. of hydrochloric acid, stopper the flask immediately to avoid loss of bromine. Shake the flask occasionally and allow to stand for 30 minutes at room temperature, keeping the flask tightly stoppered (it is necessary that a large excess of bromine be present, as shown by a bright yellow color). Add quickly 10 cc. of potassium iodide solution, stopper and shake the flask. Allow to stand for 15 minutes, shaking occasionally. Titrate the excess of iodine with 0.1 N sodium thiosulfate solution, using starch indicator. Titrate to disappearance of the blue color (a blue color develops later and should be disregarded). Calculate the amount of 0.1 N bromine combined with procaine. One cc. of 0.1 N bromide-bromate solution is equivalent to 0.00455 gram of procaine.

## RESULTS OF ANALYSIS.

*Titration after hydrolyzing with 25 cc. of 0.1 N sodium hydroxide on steam bath.*

| PROCAINE | 0.1 N BROMIDE-BROMATE REQUIRED | PROCAINE FOUND BY CALCULATION |
|---|---|---|
| *gram* | *cc.* | *gram* |
| 0.02 | 4.7 | 0.021 |
| 0.05 | 10.8 | 0.049 |
| 0.10 | 21.7 | 0.099 |

## COLLABORATIVE WORK.

*Titration of procaine after hydrolyzing on steam bath.*

| ANALYST | PROCAINE | 0.1 N BROMIDE-BROMATE REQUIRED | PROCAINE FOUND BY CALCULATION |
|---|---|---|---|
| | *gram* | *cc.* | *gram* |
| L. Jones, U. S. Food and Drug Inspection Station, Chicago, Ill. | 0.05 | 10.8 | 0.0491 |
| | 0.10 | 21.8 | 0.0999 |
| | 0.15 | 32.6 | 0.1483 |
| | 0.20 | 43.4 | 0.1975 |
| H. O. Moraw, U. S. Food and Drug Inspection Station, Chicago, Ill. | 0.05 | 10.6 | 0.0484 |
| | 0.10 | 21.6 | 0.0982 |
| | 0.15 | 32.2 | 0.1465 |
| | 0.20 | 43.6 | 0.1984 |

*Titration of procaine in hypodermic tablets after hydrolyzing on steam bath.*

| ANALYST | PROCAINE DECLARED | 0.1 N BROMIDE-BROMATE REQUIRED | PROCAINE FOUND BY CALCULATION |
|---|---|---|---|
| | *gram* | *cc.* | *gram* |
| A. W. Hanson | 0.02* (1 tablet ) | 4.7 | 0.0214 |
| | 0.04* (2 tablets) | 9.3 | 0.0423 |
| | 0.06* (3 tablets) | 14.0 | 0.0637 |
| L. Jones | 0.05* (2½ tablets) | 11.6 | 0.0528 |
| | 0.10* (5 tablets) | 23.9 | 0.1087 |
| A. W. Hanson | 0.05† (1 tablet ) | 10.2 | 0.0464 |
| | 0.10† (2 tablets) | 21.1 | 0.0960 |
| | 0.15† (3 tablets) | 32.0 | 0.1456 |
| L. Jones | 0.05† (1 tablet ) | 10.8 | 0.0491 |
| | 0.10† (2 tablets) | 21.45 | 0.0976 |

* Procaine Hypodermic Tablets. Label: Each tablet contains 0.02 gram (⅓ grain) Procaine, 0.00004 gram Adrenalin. ⅓ grain=0.0216 gram.

† Procaine Hypodermic Tablets. Label: Each tablet contains 0.05 gram (¾ grain) Procaine. ¾ grain =0.0486 gram.

## COMMENTS.

Procaine is a para-amino-benzoic acid derivative. By heating procaine with 0.1 N sodium hydroxide, the compound is decomposed, liberating the para-amino benzoate which can be titrated by bromination. In titrating the procaine, 3 molecules (6 atoms) of bromine are required, as in the cases of phenol and salicylic acid.

In case tablets are to be analyzed, it may be advisable to make up a definite quantity to a known volume, using slightly acidulated water and a volumetric flask. Any water-insoluble material can then be filtered off and aliquots taken for the titration. It is possible to obtain a representative sample of a large number of tablets in this manner. The presence of salt, which is usually used in the preparation of procaine tablets, does not interfere with the titration. A control should be run if other substances are present.

RECOMMENDATION.

It is recommended that the method submitted herewith be studied during the coming year with a view to its adoption by the association if found satisfactory.

# STUDY OF THE DISTILLATION METHOD FOR THE ESTIMATION OF SANTALOL IN SANTAL OIL.

By C. W. HARRISON (U. S. Food and Drug Inspection Station, Park Avenue Building, Baltimore, Md.).

The following methods, with notes and explanations, were sent to the collaborators:

## INSTRUCTIONS TO COLLABORATORS.

### U. S. PHARMACOPŒIA METHOD.

Acetylate the oil as directed in the U. S. Pharmacopœia[1] and into a 100 cc. Erlenmeyer flask weigh accurately about 5 cc. of the dry, filtered, acetylated oil. Pipet 50 cc. of alcoholic potash (approximately 0.5N) into the Erlenmeyer flask, measuring at the same time with the same pipet drained a similar length of time another 50 cc. portion of the alcoholic potash to carry through as a blank. Insert into the neck of the flasks containing the sample and blank, small short-necked funnels, place on a steam bath and heat until the oil is completely saponified; this will require about 1 hour. When saponification is complete, titrate the sample and blank with 0.5N sulfuric acid and phenolphthalein.

Cc. of 0.5N acid required to neutralize blank minus cc. to neutralize sample = cc. of 0.5N potassium hydroxide required to saponify the weight of oil taken. This figure is designated as "A" in the following formulae:

$$\frac{A \times 11.11}{\text{Weight of acetylated oil} - (A \times 0.021)} = \text{per cent santalol, U. S. P. method.}$$

Also calculate the saponification number of acetylated oil as follows:

$$\frac{A \times 28.06}{\text{Weight of acetylated oil.}}$$

### HARRISON DISTILLATION METHOD.

Render the flask containing the residue, after determination of the santalol by the U. S. P. method, very faintly alkaline with 1–2 drops of alcoholic potash, place on the steam bath and evaporate to a volume of about 10 cc. (this is accomplished by using suction to remove the vapors).

Carefully transfer the contents of the flask to a volatile acid apparatus of the modified Hortvet type. Make the transfer by pouring as much of the contents of the flask as possible through a funnel into the apparatus, then rinse the flask and funnel with successive small portions of dilute sulfuric acid (approximately 10 per cent by weight) until the contents of the apparatus are distinctly acid to methyl orange, 1–2 drops of which have been added to the apparatus. This should not require more than 15–20 cc. of acid. It is necessary to keep this volume small or it will delay unnecessarily the time of distillation.

[1] U. S. Pharmacopœia, IX, 1916, 296.

Then start the distillation. Most of the volatile acids will pass over in the first 150 cc. of distillate but it will require about 350 cc. of distillate to carry over the last traces of volatile acids.

Titrate the distillate with 0.5 N alkali and phenolphthalein and calculate the results as follows:

$$\frac{\text{cc. 0.5 N alkali} \times 11.11}{\text{Weight of acetylated oil} - (\text{cc. of 0.5 N alkali} \times 0.021)} = \text{per cent santalol, by distillation method.}$$

NOTES AND EXPLANATIONS OF THE METHODS.

The present U. S. P. assay of oil of santal is not applicable if the oil has been adulterated with a saponifiable oil and the distillation method is designed to show this class of adulteration.

The method is comparatively simple; the only standardized solutions required are 0.5 N sulfuric acid and 0.5 N sodium hydroxide. The other solutions used need be only approximate strength, 0.5 N alcoholic potash and 10 per cent by weight sulfuric acid.

The volatile acid apparatus is a modified form of the Hortvet apparatus which has been used in the Bureau of Chemistry for some years and was made by the glassblower. It consists of an elongated bulb, about 6½ inches long and 1½ inches in diameter, sealed at the lower end, the upper end drawn into a tube about 2½ inches long with an internal diameter about $\frac{5}{16}$ inch. This tube passes through a No. 9 rubber stopper and the upper end connects by a goose neck and rubber tube to a condenser. The No. 9 rubber stopper fits into a 2-liter Erlenmeyer flask which serves as a steam reservoir and into which the bulb, which contains the liquid to be distilled, fits.

From the side of the bulb, less than half its length from the top, a glass tube passes through the side wall and extends almost to the bottom of the bulb. This allows the steam to pass from the reservoir and bubble through the liquid in the bulb, thus carrying over the volatile acids. A small glass tube with a stop cock also passes through the rubber stopper into the steam reservoir and serves as a vent. This is left open until the distillation is well under way. A few glass beads are placed in the Erlenmeyer flask to give a uniform boiling.

A convenient way of drying the acetylated oil is to place it in a small cylinder, filled nearly to the surface of the liquid with anhydrous calcium chloride (4 mesh), and allow it to stand overnight, then filter.

Description of the four samples and their constants, as determined by the writer, are as follows:

Sample No. 1.—Oil of Santal, East India U. S. P. Fritzsche Bros., New York, N.Y.

| | |
|---|---|
| Specific gravity $\frac{25°C.}{25°}$ | 0.973 |
| Refractive index at 25°C | 1.5045 |
| Optical rotation 100 mm. at 25°C | −18.4 |
| Solubility 5 volumes 70% alcohol. Complete except slight turbidity. | |

Sample No. 2.—Santal Oil, *Oleum Santali*, U. S. P. IX, East Indian. "Distilled from genuine imported East Indian Sandalwood logs by Sharp & Dohme, Baltimore, Md."

| | |
|---|---|
| Specific gravity $\frac{25°C.}{25°}$ | 0.972 |
| Refractive index at 25°C | 1.5039 |
| Optical rotation 100 mm. 25°C | −18.2 |
| Solubility 5 volumes 70% alcohol | Complete |

Sample No. 3.—Mixture
Santal oil No. 1.....75 per cent.
Palm nut oil.....19 per cent.
Cubeb oil.....6 per cent.
Optical rotation 100 mm. tube at 25°C..... −15.0
Solubility 5 volumes 70% alcohol:.....Not complete

Sample No. 4.—Mixture
Santal oil No. 2.....80 per cent.
Cocoanut oil.....15 per cent.
Copaiba oil.....5 per cent.
Optical rotation 100 mm. tube at 25°C..... −15.0
Solubility 5 volumes 70% alcohol.....Not complete

The results of the collaborators on these four samples are given in the following table:

TABLE 1.

*Comparative results obtained by two methods.*

| SAMPLE NUMBER AND ANALYST | SAPONIFICATION NUMBER OF ACETYLATED OIL | SANTALOL U. S. P. METHOD | SANTALOL HARRISON DISTILLATION METHOD |
|---|---|---|---|
| Sample No. 1: | | *per cent* | *per cent* |
| E. K. Nelson, Bureau of Chemistry, Washington, D. C. | 196.7 | 91.4 | 86.1 |
| C. W. Harrison. | 197.6 | 92.6 | 85.3 |
| | ..... | 91.0 | .... |
| | ..... | 93.0 | .... |
| E. O. Eaton, U. S. Food and Drug Inspection Station, San Francisco, Calif. | 196.8 | 91.4 | 92.2 |
| | 199.0 | 92.7 | .... |
| C. K. Glycart, U. S. Food and Drug Inspection Station, Chicago, Ill. | 204.7 | 93.1 | 85.5 |
| | 201.6 | 91.8 | 87.2 |
| A. W. Hanson, U. S. Food and Drug Inspection Station, Chicago, Ill. | 199.3 | 92.8 | 88.4 |
| | 200.0 | 93.1 | 90.5 |
| Maximum variation..... | 204.7 | 93.1 | 92.2* |
| Minimum variation..... | 196.7 | 91.0 | 85.3 |

* Eaton did not use the distillation apparatus described in the method.

TABLE 1.—Continued.

| SAMPLE NUMBER AND ANALYST | SAPONIFICATION NUMBER OF ACETYLATED OIL | SANTALOL U. S. P. METHOD | SANTALOL HARRISON DISTILLATION METHOD |
|---|---|---|---|
| Sample No. 2: | | *per cent* | *per cent* |
| E. K. Nelson | 194.3 | 90.0 | 86.1 |
| C. W. Harrison | 192.0 | 88.9 | 84.1 |
| E. O. Eaton | 197.6 | 92.1 | 84.2 |
| | 200.3 | 93.1 | 84.2 |
| C. K. Glycart | 205.3 | 96.0 | 82.9 |
| | 200.0 | 92.4 | 87.3 |
| A. W. Hanson | 199.3 | 92.9 | 89.4 |
| Maximum variation | 205.3 | 96.0 | 89.4 |
| Minimum variation | 192.0 | 88.9 | 82.9 |
| Sample No. 3: | | | |
| E. K. Nelson | 199.4 | 92.8 | 71.4 |
| C. W. Harrison | 196.5 | 91.3 | 69.9 |
| | 197.0 | 91.4 | 70.3 |
| E. O. Eaton | 202.4 | 94.5 | 75.8 |
| | 197.5 | 91.8 | .... |
| C. K. Glycart | 205.0 | 96.5 | 73.3 |
| A. W. Hanson | 197.7 | 91.8 | 73.6 |
| Maximum variation | 205.0 | 96.5 | 75.8 |
| Minimum variation | 196.5 | 91.3 | 69.9 |
| Sample No. 4: | | | |
| E. K. Nelson | 196.2 | 91.1 | 74.2 |
| C. W. Harrison | 193.5 | 90.4 | 70.9 |
| | 194.7 | 89.6 | 73.5 |
| E. O. Eaton | 200.0 | 93.1 | 76.6 |
| | 197.0 | 91.4 | 73.5 |
| C. K. Glycart | 211.5 | 100.4 | 75.8 |
| | 208.5 | 96.8 | 75.6 |
| A. W. Hanson | 199.2 | 92.5 | 71.3 |
| Maximum variation | 211.5 | 100.4 | 76.6 |
| Minimum variation | 193.5 | 89.6 | 70.9 |

## COMMENTS BY ANALYSTS.

*E. K. Nelson.*—I believe that in pure oil the results by the proposed method must more nearly give the true percentages of santalol because the acids present in small amount in the oil (santalic and tetrasantalic acid) being of high molecular weight, would not be likely to come over with steam, but would affect the results for santalol in the official method.

On the other hand, assuming the presence of a santalol ester of one of them, (say of santalol santalated), the santalol in such an ester would probably be determined by

the distillation method only if such an ester were converted into santalol acetate in the process of acetylating. This seems probable as acetic acid must be much stronger than the acids present in the oil. On the whole, there are more points in favor of the Harrison distillation method.

*E. O. Eaton.*—Sufficient oil should be acetylized for several determinations, say 25 cc. The acetylized oil should be washed with hot water with mechanical agitation, preferably a current of air. All the anhydride is not always converted into acetic acid when the free acid is neutralized with sodium carbonate. The fused calcium chloride should be rendered anhydrous by the analyst at a temperature below red heat. Baker's C. P. anhydrous calcium chloride was found to contain considerable water. Our laboratory had no facilities to reduce the volume of hydro-alcoholic oil solution after titration, effective in less than 7 days, and so the standard 2-flask and trap method was used.

*C. K. Glycart.*—It is suggested that the directions in the Ninth Revision of the U. S. Pharmacopœia for preparing the acetylated oil be stated in definite terms: the number of washings with water; the amount of sodium carbonate test solution; also whether the excess of sodium carbonate should be removed by further washings with water.

Evaporation in the Erlenmeyer flask was found to be slow even with the aid of an electric fan (more than 10 hours). By transferring to a beaker with a large surface evaporation was rapid.

*A. W. Hanson.*—Samples 3 and 4 are evidently adulterated, as shown by the Harrison steam distillation method. It might be well to use a trap in making the steam distillation to prevent the sulfuric acid from being carried over mechanically. The residue after saponification is rather sticky and it is possible that by using a portion of alcohol it could be more readily transferred to a steam distillation apparatus.

## REVIEW OF ANALYTICAL DATA.

The results do not indicate as close agreement between the analysts as desired, but the differences were no greater than occurred when results were reported by the official method. It would seem that the collaborators obtained even greater variations when using the U. S. P. method. This is illustrated by Sample 4, where one analyst reported 100.4 per cent of santalol (U. S. P. method) and another reported 89.6 per cent by the same method. It is also evident that they did not entirely understand the details of either method and considered further explanation necessary. It appears, therefore, that if the present official method is eventually retained, further details are necessary to clarify it to insure uniform results.

Nelson points out very clearly the advantages which the distillation method possesses over the present U. S. P. method when dealing with pure oils, and the results on Samples 3 and 4 clearly show how unreliable are the results given by the U. S. P. method when assaying adulterated oils.

It is therefore evident that the present official assay method is not satisfactory, as it does not give uniform results when applied by different analysts, nor does it give reliable results with adulterated oils. The distillation method should be further studied with a view to working out further details, so that more uniform results can be obtained

by different analysts, and establishing the minimum percentage of santalol which pure oils should show when assayed by this method.

It was moved, seconded and adopted, that a representative of the association be appointed to collaborate with the Revision Committee of the United States Pharmacopœia and report progress at the next annual meeting of the association.

The meeting adjourned at 5.30 p. m. for the day.

## SECOND DAY.

# TUESDAY—MORNING SESSION.

No general report on dairy products was made by the referee.

## THE CRYOSCOPY OF MILK[1].

By Julius Hortvet (State Dairy and Food Commission, St. Paul, Minn.), *Referee on Dairy Products.*

The work included:

(1) A study of the cryoscopic method as applied to (*a*) samples of milk obtained from individual cows and herds; and (*b*), a number of series of samples consisting of milk mixed with known percentages of water.

(2) A study of the literature on the cryoscopy of milk, with special reference to (*a*) types of cryoscopes used by various investigators; (*b*) conditions under which cryoscopic tests have been carried out; (*c*) construction of thermometers; and (*d*), methods of manipulation.

The report included a fairly complete summary of the literature on the cryoscopy of milk with a critical discussion of apparatus and procedures employed by various investigators. The report concluded with a discussion of the tabulations of results obtained by collaborators and attention was called to the relationships found to exist among results obtained by various methods applied for the purpose of detecting added water. A summary was given of results obtained on 75 authentic samples. The report showed conclusively:

(1) That the cryoscopic method as applied to the examination of milk is in need of standardization. In other words, it is necessary that uniformity be secured respecting essential conditions, chiefly the following, viz; the construction of the cryoscope, the method of testing and correcting the thermometer, and the procedure.

(2) That the application of correction factors for all practical purposes may be avoided by means of a carefully standardized procedure.

(3) That results obtained by means of the apparatus and procedure described in the report indicate a narrow range of freezing-point values as a characteristic property of milk.

(4) That the cryoscopic test is reliable as a method for the determina-

[1] The complete report of the referee is not included in *This Journal* as it has previously been published in *J. Ind. Eng. Chem.*, 1921, 13: 198.

tion of added water in amount far below 10 per cent. When the freezing point of the original whole milk is known, results are obtainable to within an error not far from 0.5 per cent and when the freezing point of the original milk (*e. g.*, herd milk) is unknown, the addition of water may safely be reported in amount as low as 3 per cent.

## RECOMMENDATION.

It is recommended that the following cryoscopic method for the examination of milk be given further study with a view to its adoption as official:

### CRYOSCOPE.

A cylindrical-shaped Dewar flask of 1 liter capacity, and 28 cm. internal depth, surrounded by a metal casing, is tightly closed by means of a large cork of about 4 cm. thickness. Through the center of the cork is fitted tightly a medium thin-walled glass tube, 255 mm. in length by 33 mm. outside diameter. At one side of the cork is inserted a narrow copper inlet tube, the lower end of which is formed into a perforated loop near the bottom of the flask. At the opposite side is a metal tube of T-shape construction and 6 mm. internal diameter, intended to afford escape for vapors, and also for the introduction of volatile fluid into the apparatus. At the back portion of the cork is fitted a control thermometer having a scale range of +20° to −30° C., with the bulb extending nearly to the bottom of the flask. The freezing test tube is of thin glass, about 250 mm. in length by 30 mm. outside diameter, and fits closely into the larger tube which is sealed into the cork. In the rubber stopper of the freezing tube is fitted the standard thermometer. The thermometer is constructed of sufficient length to enable the insertion of the bulb near the bottom of the freezing test tube and at the same time allow complete exposure of the scale above the stopper. At the right-hand side of the thermometer a stirring device made of noncorrodable low conductivity metal is fitted into the stopper through a short section of metal tubing. The lower end extends nearly to the bottom of the test tube and is provided with a loop around the outside of which are a number of pointed projections. At the left of the thermometer is a freezing starter attachment inserted through an opening in the stopper formed by means of a short section of metal tubing. This device consists of a noncorrodable metal rod, at the lower end of which is a 10 mm. length opening for the purpose of carrying a small fragment of ice. At one side of the cryoscope is installed an air-drying arrangement which consists of a Folin absorption bulb inserted through a tightly fitting stopper and extending nearly to the bottom of a large size test tube. A short section of glass tubing is inserted through a second opening in the stopper and is connected with the vaporizing tube which enters the cryoscope. Sulfuric acid is poured into the drying tube to a level slightly above the inner bulb. At the opposite side of the apparatus is arranged a drain tube for the purpose of conducting vapors away from the operator. By means of a pressure and suction pump dry air may be forced into the apparatus at a suitable rate and the mixed vapors conducted out through the base of the drain tube into the sink. An adjustable lens is mounted in a suitable position in front of the thermometer for the purpose of magnifying the scale.

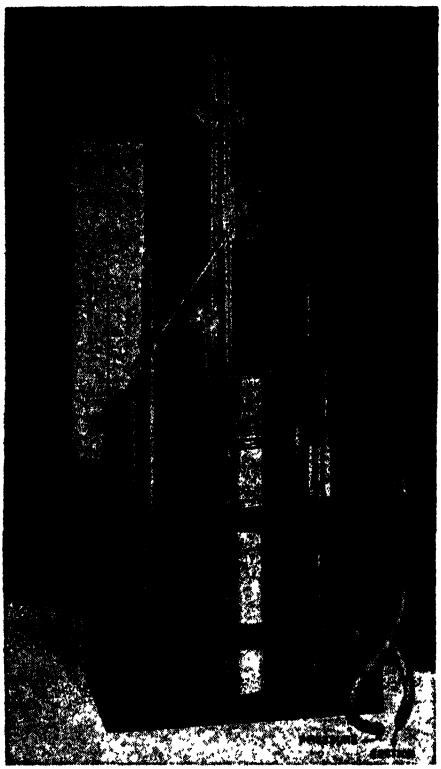

FIG. 1 HORTVET CRYOSCOPE.

THERMOMETER.

The standard thermometer designed especially for testing milk is a solid-stem instrument measuring a total length of 58 cm., with a scale portion measuring 30 cm. The total scale range is 3°C., from +1° to -2°C., each degree division being subdivided into tenths and hundredths. The length of a degree division approximates to 1 decimeter, thus making the smallest subdivisions of such magnitude as to enable easy observation and readings estimated to 0.001°C. The thermometer should be carefully standardized and calibrated in comparison with a Bureau of Standards tested instrument.

The control thermometer should be tested in a bath of melting crushed ice for the purpose of determining whether the zero mark on the scale is fairly correct to within a small fraction of a degree.

DETERMINATION.

Insert a small caliber funnel-tube into the vertical portion of the T-tube at one side of the apparatus and add 400 cc. of ether, previously cooled to 10°C. or lower. Close the vertical tube by means of a small cork and connect the pressure pump to the inlet tube of the air-drying attachment. Adjust the pump so as to pass air through the apparatus at a moderate rate, which may be judged by the agitation of the sulfuric acid in the drying tube. Continuous vaporization of the ether will cause a lowering

of the temperature in the flask, from ordinary room temperature to 0°C. in about 8 minutes. Continue the temperature lowering until the control thermometer registers near -3°C. At this stage, by lowering a narrow-gauge, graduated glass tube into the ether bath, then closing the top by means of the forefinger and raising to a suitable height, an estimate can be made of the amount of ether necessary to add to restore the 400 cc. volume. When the apparatus has been cooled to the proper temperature, an additional 10–15 cc. of ether is on an average sufficient for each succeeding determination. Measure into the freezing test tube 30–35 cc. of boiled distilled water, cooled to 10°C. or lower. Enough water should be added to submerge the thermometer bulb. Insert the thermometer, together with the stirrer, and lower the test tube into the larger tube. A small quantity of alcohol, sufficient to fill the space between the two test tubes, will serve to complete the conducting medium between the interior of the apparatus and the liquid to be tested. A sufficiently tight connection between the inner and outer tubes is afforded by means of a narrow section of thin-walled rubber tubing. Keep the stirrer in steady up-and-down motion at a rate of approximately one stroke each 2–3 seconds, or even at a slower rate, provided the cooling proceeds satisfactorily. Maintain the passage of air through the apparatus until the temperature of the cooling bath reaches -2.5°C., at which time the top of the mercury thread in the standard thermometer usually recedes to a position in the neighborhood of the probable freezing point of water. Maintain the temperature of the cooling bath at -2.5°C. and continue the manipulation of the stirrer until a supercooling of the sample of 1.2°C. is observed. As a rule, by this time the liquid will begin to freeze, as may be noted by the rapid rise of the mercury thread. Manipulate the stirrer slowly and carefully three or four times as the mercury column approaches its highest point. By means of a suitable light weight cork mallet tap the upper end of the thermometer cautiously a number of times, until the top of the mercury column remains stationary a couple of minutes. Taking necessary precautions to avoid parallax, observe the exact reading on the thermometer scale and estimate to 0.001°C. When the observation has been satisfactorily completed, make a duplicate determination, then remove the thermometer and stirrer and empty the water from the freezing tube.

Rinse out the test tube with about 25 cc. of the sample of milk, previously cooled to 10°C. or lower, measure into the tube 35 cc. of the milk, or enough to submerge the thermometer bulb, and insert the tube into the apparatus. Maintain the temperature of the cooling bath at 2.5°C. below the probable freezing point of the sample. Make the determination on the milk, following the same procedure as that employed in determining the freezing point of water. As a rule, however, it is necessary to start the freezing action in the sample of milk by inserting the freezing starter, carrying a fragment of ice, at the time when the mercury column has receded to 1.2°C. below the probable freezing point. A rapid rise of the mercury column results almost immediately. Manipulate the stirrer slowly and carefully two or three times while the mercury column approaches its highest point. Complete the adjustment of the mercury column in the same manner as in the preceding determination; then, avoiding parallax, observe the exact reading on the thermometer scale and estimate to 0.001°C. The algebraic difference between the reading obtained on the sample of water and the reading obtained on the sample of milk represents the freezing-point depression of the milk.

To deduce the percentage of added water from the determined freezing point, use Winter's table[1] or the scale accompanying the cryoscope. The percentage of added water (W) may also be calculated as follows:

$$W = \frac{100\ (T-T')}{T'} \text{ in which}$$

[1] *Chem. News*, 1914, **110**: 283.

T = the average freezing point of normal milk (-0.550°C.); and
T′ = the observed freezing point on a given sample.

No report on moisture in cheese was made by the associate referee.

## DETERMINATION OF FAT IN MALTED MILK.

By J. T. KEISTER (Bureau of Chemistry, Washington, D. C.).

The determination of fat in malted milk by the official Roese-Gottlieb method and modifications thereof, has been the subject of more or less collaborative study by the association since 1916 with wide variations in results. The writer, among others, last year also tested a direct ether extraction process which produced the most concordant results so far obtained. This method is objectionable, however, because of the time consumed in its operation and, for this reason, attention has been directed to a further study of a suitable modification of the official Roese-Gottlieb method. Aside from the wide variations in the results heretofore obtained by the official method, it would also appear that the Roese-Gottlieb method gives figures slightly below the actual percentage of fat present.

The work here reported consists of a comparison of the regular official method with the same method omitting the use of ammonia. While the results are not entirely satisfactory, it is believed they are sufficient to warrant further consideration of this proposed modification.

*Comparative results on the determination of fat.*

(Results calculated to a water-free basis.)

| OFFICIAL ROESE-GOTTLIEB METHOD | OFFICIAL ROESE-GOTTLIEB METHOD OMITTING AMMONIA |
|---|---|
| *per cent* | *per cent* |
| Sample A | |
| 8.655 | 8.526 |
| 8.577 | 8.676 |
| 8.569 | 8.680 |
| 8.469 | 8.710 |
| ..... | 8.750 |
| Average.....8.574 | 8.668 |
| Sample B | |
| 7.568 | 8.032 |
| 7.829 | 7.923 |
| 7.920 | 7.763 |
| 7.998 | 7.935 |
| 7.750 | ..... |
| Average.....7.813 | 7.913 |

It will be noted that the results in both cases show an average about 0.10 per cent higher by the method in which the use of ammonia was omitted. It is also noted that more concordant results were obtained in the case of Sample A than with Sample B, which would indicate a greater difficulty in extracting the fat from some brands of malted milk than others.

Results obtained last year on a collaborative sample were not satisfactory by the method omitting the use of ammonia, because of the formation of an emulsion which almost filled the extraction tube. It is believed that this condition was principally due to the proportions of water and alcohol used—about 8 cc. of water to 5 cc. of alcohol. In the work here reported, 10 cc. of water and 10 cc. of alcohol were used and, in some cases, when the third extraction was made if an emulsion was inclined to form, a further addition of 1 or 2 cc. of alcohol was made with satisfactory results.

To obtain the most satisfactory results with this modification the following points should be observed:

1. Use about a 1-gram sample, add 10 cc. of water and rub thoroughly with a glass rod in a small beaker until all visible particles of the powder have disappeared and a homogeneous emulsion is formed. This is very necessary; otherwise, when the alcohol is added, lumps are formed which it is difficult or impossible to break up, thereby rendering the extraction incomplete. This condition is probably due to the fact that dextrine is soluble in water but insoluble in alcohol. Therefore, it must be brought in complete solution with the water before the addition of alcohol to avoid the formation of lumps.

2. It is necessary to shake the extraction tube longer (at least 1 minute) than is required in the case of milk powder or other milk product.

3. A third extraction is always necessary.

4. A slight amount of insoluble material is sometimes obtained in the third extraction, which must be determined and the proper correction made.

## CONCLUSION.

The fat is extracted from malted milk with greater difficulty than from any other milk product, but it is believed that by the observance of the above-noted points and with a little experience the Roese-Gottlieb method without the use of ammonia will completely extract the fat from this product.

# REPORT ON FATS AND OILS.

By R. H. KERR (Bureau of Animal Industry, Washington, D. C.), *Referee.*

The work consisted of a comparative study of the Hanus and Wijs methods for determination of the iodine number. As the relative merits of these two methods have been the subject of more or less controversy, and as most of the comparative tests recorded have been made by some one previously familiar with one or the other of the two methods, the making up of the solutions was entrusted to an assistant who was not familiar with either method but who was thoroughly fitted by training and experience to carry out the work. His report is of interest and is as follows:

## COMPARISON OF THE WIJS AND HANUS METHODS.

(Analyst, A. L. MEHRING.)

The Wijs solution was prepared by mixing equivalent amounts of solutions of chlorine and iodine in glacial acetic acid. The chlorine solution was prepared by passing pure, dry chlorine into glacial acetic acid. The iodine solution was prepared by dissolving 15 grams of iodine in 600 cc. of glacial acetic acid. The strength of both was determined by titration against a standard sodium thiosulfate solution. A sufficient amount of iodine solution for 1 liter of Wijs solution was then mixed with its exact equivalent of chlorine solution and made up to a volume of 1 liter with glacial acetic acid. The Hanus solution was made according to the official method of the association. Two portions of each fat or oil were run in duplicate by the official method with each solution. Manipulation was exactly the same in each case except for the difference in time allowed for absorption, 15 minutes for the Wijs and 30 minutes for the Hanus solution. Following are the results:

TABLE 1.

*Iodine numbers obtained by the Wijs and Hanus methods.*

(Analyst, A. L. Mehring.)

| METHOD | CORN OIL | COTTONSEED OIL | HYDROGENATED TALLOW | PURE LARD | OLIVE OIL |
|---|---|---|---|---|---|
| Wijs..... | 119.69 | 107.97 | 37.97 | 61.81 | 83.77 |
| Wijs..... | 122.01 | 107.51 | 38.06 | 61.16 | 83.47 |
| Hanus..... | 118.54 | 105.11 | 39.31 | 62.43 | 80.52 |
| Hanus..... | 118.58 | 105.52 | 39.26 | 61.75 | 80.26 |

The results obtained with the Hanus solution showed a slightly less variation than those with the Wijs. The numbers obtained for oils giving the highest values were uniformly higher with the Wijs than with the Hanus solution. The greater length of time required for the absorption of the Hanus solution is a factor only when a single or very small number of determinations is made at the same time. The amount of labor involved in generating and washing the chlorine is a disadvantage in making up the Wijs solution, inasmuch as the corresponding bromine for the Hanus solution may easily be obtained ready for use.

Comparative tests were also made by an experienced analyst who was thoroughly familiar with the determination of the iodine number by the Hanus method. The results obtained are as follows:

TABLE 2.

*Iodine numbers obtained by the Wijs and Hanus methods.*

(Analyst, J. B. Martin.)

| DESCRIPTION OF SAMPLE | HANUS | WIJS |
|---|---|---|
| Lard | 60.33<br>59.91 | 63.14<br>63.36 |
| Hydrogenated beef fat | 39.15<br>39.04 | 37.67<br>37.62 |
| Olive oil | 81.51<br>80.83 | 83.45<br>83.69 |
| Cottonseed oil | 105.34<br>105.71 | .....<br>..... |
| Corn oil | 121.47<br>121.68 | .....<br>..... |

RECOMMENDATION.

It would appear from these results that the Hanus and Wijs methods give fairly comparable results. If the results by either method are taken as a standard, those obtained by the other method would have to be regarded as somewhat erratic. The results show no reason for regarding either method as superior to the other. It is recommended, therefore, that the Hanus method be continued as the official method with the Wijs method as the tentative method of the association, as at present.

## REPORT ON BAKING POWDER.

By G. H. MAINS (Bureau of Chemistry, Washington, D. C.), *Referee.*

The work was upset by the midseason resignation of the referee, H. E. Patten, and some time elapsed before the writer was asked to look after the work for the remainder of the year.

The recommendations for 1919 called for a further study of the electrolytic method for the determination of lead, of minor details of the fluoride method, and of methods for the determination of the neutralizing strength of baking acids. The work has been necessarily limited to collaborative lead determinations, using the electrolytic method, and to a preliminary survey of methods for determining neutralizing values.

## DETERMINATION OF LEAD BY THE ELECTROLYTIC METHOD.

The samples sent to the collaborators were made up to specifications from specially purified commercial materials, prepared through the courtesy of E. W. Thornton of the R. B. Davis Baking Powder Company, Hoboken, N. J. One set consisted of a straight mono-calcium phosphate powder, the other of a combination phosphate and sodium aluminium sulfate powder. Each type contained 25 per cent by weight of sodium bicarbonate, and the total lead content (added in the form of sulfate) was 50 parts per million.

Collaborators were requested to use the tentative electrolytic method[1] except that they were given the option of substituting a colorimetric for a gravimetric determination in the final step.

Relatively few of the collaborators presented reports. The results obtained are shown in the following table. The figures for each separate determination are presented, the amount of lead found being reported as the nearest whole number of parts per million:

*Determination of lead in baking powder by the electrolytic method.*

| COLLABORATOR | LEAD FOUND BY ANALYSIS | |
|---|---|---|
| | Phosphate powder (Lead content: 50 parts per million) | Combination phosphate-sodium aluminium sulfate powder. (Lead content: 50 parts per million) |
| | *parts per million* | *parts per million* |
| A. H. Fiske and A. L. Thayer, Rumford Chemical Works, Providence, R. I. | 9<br>9 | 15<br>15 |
| A. Malmstrom, Wilckes-Martin-Wilckes Co., Camden, N. J. | 26<br>26 | 17<br>26<br>32<br>32 |
| W. E. Stokes and D. J. Kaplan, Royal Baking Powder Co., New York, N. Y. | 23 | 42<br>45 |

A. H. Fiske used a modification of the method in which the organic matter was destroyed by ignition with magnesium nitrate before the preparation of the solution for electrolysis. A. Malmstrom found that with the combination phosphate-sodium aluminium sulfate powder a more satisfactory medium for electrolysis was obtained by using 50 grams of the sample in place of the 100 grams specified. Stokes and Kaplan recommend that the period of electrolysis be extended from 8 to 15 hours.

[1] *Assoc. Official Agr. Chemists, Methods.* 2nd ed., 1920, 287.

In general, the results of each collaborator fall short of the actual lead content, and do not agree very well with those of other collaborators. However, it will be noted that each collaborator, when running his samples by his own particular technique, secured consistent results. This would seem to indicate that the method requires a closer regulation of conditions during electrolysis, especially with regard to acidity of solution, in order to insure the deposition of all of the lead during the electrolysis. From theoretical electro-chemical considerations, in order to obtain the last traces of lead, the hydrogen-ion concentration of the solution for electrolysis should be reduced as low as practicable, and means taken to increase the hydrogen over-voltage at the cathode. For this purpose a study of the substitution of other metals, particularly nickel and chromium, for platinum as the cathode is suggested.

## METHODS FOR THE DETERMINATION OF THE NEUTRALIZING STRENGTH OF BAKING ACIDS.

The present official method[1] for acidity of cream of tartar and its substitutes does not give consistent results when applied to the determination of the neutralizing strength of commercial acid phosphates, and it was recommended in 1919 that a study be made of this question. A number of inquiries have been received during the past two years for standard methods for the determination of the neutralizing value, and several of the manufacturers have expressed a willingness to cooperate in this study. A general survey of the methods that have been submitted shows them to be quite similar except for some details which cause the big differences in the values obtained.

The writer has obtained fairly consistent results with certain types of mono-calcium phosphates, using the following modification of the available methods:

Weigh 0.84 gram of the phosphate into a 250 cc. beaker, add 125 cc. of water, and a large amount of phenolphthalein indicator (10–15 drops of the ordinary solution). Titrate with 0.5 N sodium hydroxide to a faint pink; heat to boiling; boil for 1 minute, and continue titrating while hot till a permanent pink is reached. The total reading, multiplied by 5, equals the neutralizing value in terms of parts of $NaHCO_3$ per 100 parts of phosphate.

Other methods call for the addition of salt solution to prevent hydrolysis, the addition of sodium hydroxide in excess and back titration with hydrochloric acid, or the use of sodium carbonate instead of sodium hydroxide. The ionization constants of phosphoric acid do not indicate any necessity for protection against hydrolysis, but such details and others that may be proposed should form part of the study for the coming year.

[1] *Assoc. Official Agr. Chemists, Methods.* 2nd ed., 1920, 281.

## PRESENT STATUS OF BAKING POWDER METHODS.

The writer must say a word of appreciation for the large amount of valuable work on baking powder that the former referee, H. E. Patten, together with his collaborators, has done during the past four years. While there will always be room for improvement, the baking powder methods are today in very good shape. There has been especially valuable collaboration on the part of the larger manufacturers of baking powders and ingredients, both in the development of methods and in the improvement of the purity of their products. Valuable work has also been carried on by a number of official and commercial chemists. It is to be hoped that the incoming referee will enjoy the same helpful co-operation.

## RECOMMENDATIONS.

It is recommended—

(1) That a further study be made of the Chittick method[1] for the determination of lead in baking powders with the view to establishing it as a tentative method.

(2) That a study be made of the details of the electrolytic method for the determination of lead in baking powder with special reference to the acidity conditions during electrolysis.

(3) That a study be made of methods for the determination of the neutralizing strength of baking acids.

(4) That the paper entitled, "Determination of Total Carbon Dioxide in Baking Powder", presented by C. S. Robinson, and given below, be referred to the referee on baking powders and baking chemicals for study of the method contained therein.

# DETERMINATION OF TOTAL CARBON DIOXIDE IN BAKING POWDER[2].

By C. S. ROBINSON[3] (Agricultural Experiment Station, E. Lansing, Mich.).

The Association of Official Agricultural Chemists recognizes but one type of method for the determination of total carbon dioxide in baking powder, *i. e.*, the absorption type. Two variations, Knorr's[4] and Heidenhain's[5] have been adopted as official while any one which gives accurate results with calcite may be included in the class of tentative methods. The object of the present article is to call attention to certain defects in

[1] *J. Assoc. Official Agr. Chemists*, 1920, 4: 218.

[2] Journal Article No. 17 from the Chemical Laboratory of the Michigan Agricultural College Experiment Station. Published by permission of the Director of the Agricultural Experiment Station.

[3] Presented by A. J. Patten.

[4] *Assoc. Official Agr. Chemists, Methods.* 2nd. ed., 1920, 277.

[5] *Ibid.*, 279; *J. Am. Chem Soc.*, 1896, 18: 1.

the descriptions of these methods and also to suggest the adoption of another type of method which possesses marked advantages over those at present in vogue.

In the absorption type of method the carbon dioxide is liberated from the sample by dilute acid and is swept, by means of a current of air, through vessels charged with alkali which absorb it. The increase in the weight of the contents of the vessels is the measure of the carbon dioxide absorbed.

The apparatus consists of the following parts:

(1) A vessel for removing carbon dioxide from the air current used to expel the carbon dioxide produced from the sample.

(2) A reaction vessel into which the sample is weighed and in which it is decomposed. This vessel is fitted with a dropping funnel for introducing the acid and a condenser to minimize the escape of water vapor.

(3) A device for drying the stream of air and carbon dioxide.

(4) The absorption vessel or vessels.

(5) A drying arrangement to prevent the backward diffusion of moisture into the absorption vessels.

(6) An aspirating device.

The details of the apparatus are capable of limited variation. A tower of soda lime may be used for (1) or any of the usual forms of drying tubes filled with the same material or a wash bottle filled with a strong solution of alkali. Calcium chloride U-tubes or washing bottles filled with concentrated sulfuric acid may be used for (3). The carbon dioxide may be absorbed in potash bulbs containing a solution of potassium hydroxide or in U-tubes or other vessels filled with soda lime. Either of the devices used for (3) may also be used for (5) while a mechanical pump, water pump or aspirator bottle may serve to draw the air through the apparatus. Any of these various forms may be used interchangeably, as described, provided suitable precautions are observed. The two arrangements prescribed in the official methods are in reality but two variations of the above-described units.

The following criticisms of the official methods seem to be justified from the author's experience. Since the methods are essentially alike, differing only in the details of the set-up, it seems unnecessary to differentiate between them to the extent of describing them separately. The real fault, however, lies in the directions for the use of Knorr's apparatus. These are not only entirely inadequate but, at least in the writer's experience, positively misleading in one or two respects. Thus, it is prescribed that the acid used to decompose the sample shall be hydrochloric acid having a density of 1.1. The writer's experience has been that acid of this concentration invariably gave off hydrochloric acid gas which was absorbed in the absorption vessels, giving high

results. A wash bottle containing silver nitrate placed ahead of the absorption train always showed a deposit of silver chloride. Possibly the boiling of the liquid in the flask and the strength of the air current can be so regulated as to overcome this difficulty but it is so much easier to dilute the acid with an equal volume of water to a concentration which can be used with perfect safety that it seems inadvisable to maintain the original directions.

The directions for expelling the carbon dioxide are equally misleading. The rate of aspiration recommended is "about 2 bubbles per second" and the length of time required to free the apparatus from carbon dioxide is defined by the direction to "allow the apparatus to cool with continued aspiration" after boiling the liquid for a few minutes "after water has begun to condense". With the apparatus used by the writer, this period would embrace about 15 to 20 minutes which is entirely insufficient. The rate of aspiration is also indefinite as potash bulbs have been connected in a series in the same train which showed rates of aspiration varying through a range of 100 per cent or more when judged by the above standard.

But little fault can be found with Heidenhain's modification except that the original description of his apparatus and procedure, published a quarter of a century ago, is still adhered to in spite of the fact that some of it is obsolete.

By using Heidenhain's technique and general form of apparatus, excellent results can be obtained and, in fact, the conclusion seems justified that, irrespective of the apparatus used, the reliability of the results varies directly with the closeness with which his directions are followed. The writer has found that with but few exceptions all of the precautions advised by Heidenhain must be taken if one is to obtain reliable results by the absorption method. The exceptions for ordinary work are the inclusion of the absorption vessels in a glass case and the making of corrections for the temperature and pressure at the time of weighing.

The writer would suggest, however, the use of a flowmeter for controlling the rate of aspiration. He used the type adopted by the American University Experiment Station[1] which can be made by any one having an elementary knowledge of glass blowing. The greatly increased ease and accuracy with which one can control the process makes the time required for its manufacture well spent. Too much care can not be taken in controlling this factor in the method under discussion. By careful observation, the writer found that a difference in rate of less than 5 cc. per minute may cause appreciable errors in the results. With a flowmeter connected in the train next to the aspirating bottle one can tell at a glance at any time just how fast the aspiration

[1] *J. Ind. Eng. Chem.*, 1919, 11: 623.

is proceeding without taking the time to make the necessary measurements required to ascertain the rate by Heidenhain's method.

The official standard of 3 liters of air as a requisite amount to remove completely the carbon dioxide is not a safe one. Neither can the amount be ascertained for any given apparatus, as Heidenhain has suggested, without adding a factor of safety which makes the method of aspirating to constant weight preferable. With the apparatus employed by the writer, 3 liters were about the minimum amount of air required to remove the usual quantities of carbon dioxide generated but at times as high as 6 liters of air had to be drawn through before the weight of the absorption vessels became constant. If a figure for constant use had been selected it would have been necessary to choose about 6 liters as the amount to be used. It is quicker to interrupt the aspiration at the end of the third liter and after the passage of each liter thereafter to make a weighing than to always pass 6 liters of air through the apparatus and call the removal complete.

Another precaution which should not be neglected when using soda lime for absorbent purposes is to have some calcium chloride in the tube where the air enters and more where it leaves. In order to absorb the carbon dioxide efficiently, soda lime must be moist and this moisture must be prevented from escaping. In fact, the writer has found that, as Heidenhain has stated, there is little danger of a loss of carbon dioxide through its being swept through two soda-lime tubes but there is a real danger of moisture being carried from the last soda-lime tube if insufficient calcium chloride is present or if the safe rate of aspiration is exceeded.

By attending to these precautions, results have been obtained which leave nothing to be desired from the standpoint of accuracy. The writer has tried both Heidenhain's apparatus, as originally described (with the exception of the condenser), and an arrangement in which his long calcium chloride U-tubes were replaced by the more convenient and common potash bulbs containing sulfuric acid, giving in effect a modified Knorr set-up. This makes a more convenient arrangement and one composed of units available in any well-equipped laboratory.

In the light of experience with the present official methods, the writer feels that it would be advisable to eliminate that portion of them dealing with the determination of total carbon dioxide in baking powder and to substitute the following:

### TOTAL CARBON DIOXIDE.

*Absorption Method.*

#### REAGENTS.

(a) *Potassium hydroxide solution.*—Dissolve 25 grams of potassium hydroxide in 50 cc. of water.

(b) *Soda lime.*—Granulated to pass a sieve having 9–12 meshes to the inch and freed from dust by sifting.

(c) *Calcium chloride.*—Granulated to pass a sieve having 9–12 meshes to the inch, sifted to free it from dust, dehydrated at 200°C. and saturated with carbon dioxide before use.

(d) *Sulfuric acid.*—Ordinary concentrated acid (sp. gr. 1.84).

(e) *Approximately 10% hydrochloric acid.*

APPARATUS.

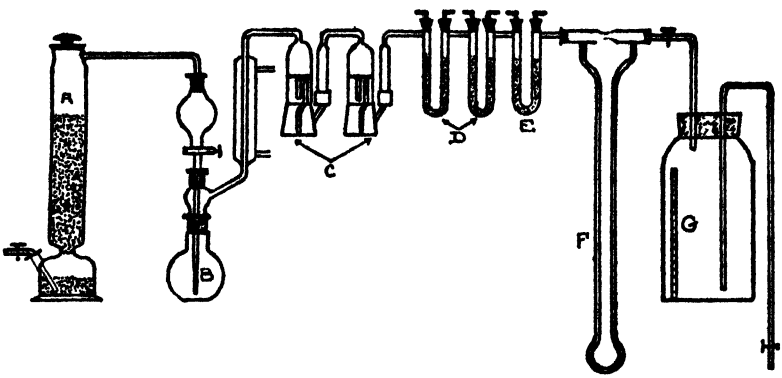


FIG. 1. APPARATUS FOR THE DETERMINATION OF CARBON DIOXIDE.

This consists of a flask *B*, Fig. 1, of 100–200 cc. capacity fitted with a dropping funnel, the stem of which reaches to the bottom of the flask and an outlet tube forms the inner tube of a reflux condenser. The stem of the dropping funnel should be drawn out to a tip and be bent upwards to prevent the advent of gas. All joints should be ground.

Standard U-tubes for holding solid absorbents (calcium chloride and soda lime) and potash bulbs of any suitable type such as Geissler, Gomberg or Liebig, for holding liquid absorbents (sulfuric acid and potassium hydroxide solution).

An aspirating device, such as a bottle arranged as shown in the figure, a suction pump or a mechanical vacuum pump.

A flowmeter having a range up to 50 cc. per minute is desirable. Otherwise, a graduated cylinder and watch must be used to measure the rate of aspiration.

Burners, ringstands, etc.

A drying tower filled with soda lime may be used for purifying the air which expels the carbon dioxide but either of the means used for absorbing the carbon dioxide from the sample itself may be substituted for it.

The general arrangement of the parts is shown in the sketch but it is to be understood that sulfuric acid and calcium chloride in the appropriate containers may be used interchangeably for the purpose of absorbing moisture, while either soda lime or potassium hydroxide solution may serve as absorbents for carbon dioxide. As shown in Fig. 1, *A* is the tower filled with soda lime for removing carbon dioxide from the air used in expelling the generated carbon dioxide into the absorbing apparatus; *B* is the reaction flask; *C* represents the potash bulbs containing sulfuric acid used to dry the mixture of air and carbon dioxide (two units should always be used, one to take care of the bulk of the moisture which comes through the condenser and a second to remove any traces which may escape the first one); *D* is the carbon dioxide absorbing apparatus; *E* is a drying tube to prevent the backward diffusion of moisture into *D*; *F* is a flow-

meter; and *G* an aspirating device. The bottle is graduated to read in half liters and should have a capacity of 6 liters or more.

Precautions should be taken with the absorbing mechanism to prevent the escape of moisture and to insure complete absorption of the carbon dioxide. If soda lime is used it should be contained in two tubes. In the first one, at the end where the carbon dioxide enters, about 1 inch of calcium chloride should be placed. The rest of this tube is filled with soda lime, as is the arm of the second tube adjacent to it. The arm of the latter by which the air leaves is filled with calcium chloride. If potash is used as the absorbent, the bulbs should either be supplied with an attached calcium chloride tube or if one of the older types is used, a second unit filled with sulfuric acid which is weighed before and after each determination should be provided.

DETERMINATION.

In order to find the allowable rapidity of the air current employed during the determination, proceed as follows: Charge the apparatus exactly as for an analysis, leaving out the carbonate. Begin to aspirate at the rate of about 50 cc. per minute. After 2 liters have been aspirated, weigh the absorption vessels. If they have lost weight, repeat the experiment with a rate of 40 cc. per minute, and so on until the weight of the vessel remains constant. If the work has been properly conducted the first unit will have lost just as much as the second will have gained. In making actual analyses do not exceed the safe speed thus found.

Weigh the absorption vessels after having opened them momentarily to equalize the air pressure. Connect them in place in the apparatus and test the tightness of the joints by closing the inlet to *A*, with all intermediate cocks open, carefully opening the cock between *F* and *G*, *G* being filled with water and in equilibrium with the outlet cock already open. If there is no leak, the liquid in the flowmeter will shortly indicate no movement of air. Then close the cock on *C* adjacent to *B* and introduce the sample into the latter, which should be dry. Replace the dropping funnel and put into it a sufficient excess of 10% hydrochloric acid so that it may be boiled in the flask without danger of cracking the glass. Connect the drying tower, *A*, with the rest of the apparatus and carefully open the cock between *B* and *C*. When the flowmeter shows the passage of no more air, admit the acid slowly into the flask, observing the flowmeter to see that the carbon dioxide is not evolved too rapidly. When all of the acid has run from the funnel into the flask, close the stop-cock in the stem of the funnel, start the water in the condenser, and heat the flask with a small flame until no more gas is evolved. (The use of sulfuric acid wash bottles, as shown in Fig. 1, for drying the gas instead of calcium chloride tubes aids in controlling this operation as the carbon dioxide at times constitutes such a high percentage of the gas entering the train that there is no passage of air through the flowmeter.) Then with a small flame under the flask to keep the liquid at the boiling point, open the cock in the dropping funnel enough to permit air to be aspirated through the apparatus at about half the rate found to be safe. After the bulk of the carbon dioxide has been expelled, increase this rate to the maximum allowable speed. When 3 liters have passed through, as indicated by the marks on the aspirator bottle, close the cock between *F* and *G* and those on the absorbing vessels. Remove and weigh the latter and replace them in the train. Pass another liter of air through and weigh again, repeating the process until there is no net gain after passing a liter of air through the apparatus, *i. e.*, until one tube gains just as much as the other loses.

The net gain in weight of the absorption vessels is due to carbon dioxide and its percentage of the sample is calculated in the usual manner.

But however much may be said of this method from the standpoint of accuracy it must be admitted that it is cumbersome and time-con-

suming. As a substitute for it a method is now proposed which requires but one piece of apparatus into which the sample is weighed and in which it is decomposed and the volume of carbon dioxide is measured. It may be allowed to stand indefinitely and then used at once without further preparation than the greasing of the stop-cocks.

The number of analyses which can be made in a given time is determined almost entirely by the rapidity with which the analyst can weigh samples, the time required for the determination being a matter of minutes. It is easily possible to make a complete estimation of the total carbon dioxide in a baking powder by this method in about 5 minutes, including weighing the sample, decomposing it, measuring the carbon dioxide liberated and calculating the results.

The accuracy of the method is at least equal to that of the more complicated absorption methods. Because of its greater simplicity, the fact that there are fewer weighings to make and in general less possible sources of error it is essentially more accurate than the methods at present in vogue.

As originally designed[1], it was applicable only to the analysis of carbonates in solution but in the modified form later described[2] it can be used equally well for solid material. The apparatus is shown in Fig. 2.

It consists of a 10 cc. buret having the upper 2 cc. graduated in 0.02 cc. and the remaining 8 cc. graduated in 0.05 cc. The upper end of this buret is closed by a 3-way stop-cock having one arm bent as illustrated and the other one sealed to a cup holding 5–10 cc. graduated to 5 cc. in 0.5 cc. The lower end of the buret is sealed to a bulb of such size that the whole apparatus will have a capacity of 50 cc. from the stop-cock *A* to a mark between the bulb and the stop-cock *B*. The openings in the stop-cocks *B* and *C* should be large, as mercury is forced through them.

The stopper *D* should be as close to *B* as convenient in order to reduce the space above it and the total capacity of the stopper and right-hand tube *F* to which the stopper is attached should be about 5 cc. The stopper should be hollow and the end should be open. It should be set at right angles to *F*.

The lower outlet of *C* is attached by a piece of heavy-walled suction tubing to a leveling bulb filled with mercury.

The following is the technique employed in making a determination: For materials so high in carbon dioxide that a sample of less than 500 mg. will liberate not over 10 cc. of gas the hollow stopper serves as a weighing bottle and the material is weighed into it, the tube being filled with mercury up to the mouth of *D* to reduce the air space which must subsequently be evacuated. For carbonate-poor substances, the sample is weighed into the tube *F*, being introduced by means of a test-tube funnel.

The whole apparatus except the right-hand tube *F* between *B* and *C* (but including the right-hand hole in *C*) is then filled with mercury. With the stop-cock *A* closed and the connection open between *B* and *C* through the tube *E*, the leveling bulb is lowered to such a position that the mercury level drops below *C*, evacuating the buret, bulb, etc. (This is done conveniently by means of a heavy cord of proper length attached to the bulb by one end and by the other to the support holding the apparatus.)

[1] *J. Biol. Chem.*, 1917, **30**: 347.
[2] *Soil Science*, 1920, **10**: 41.

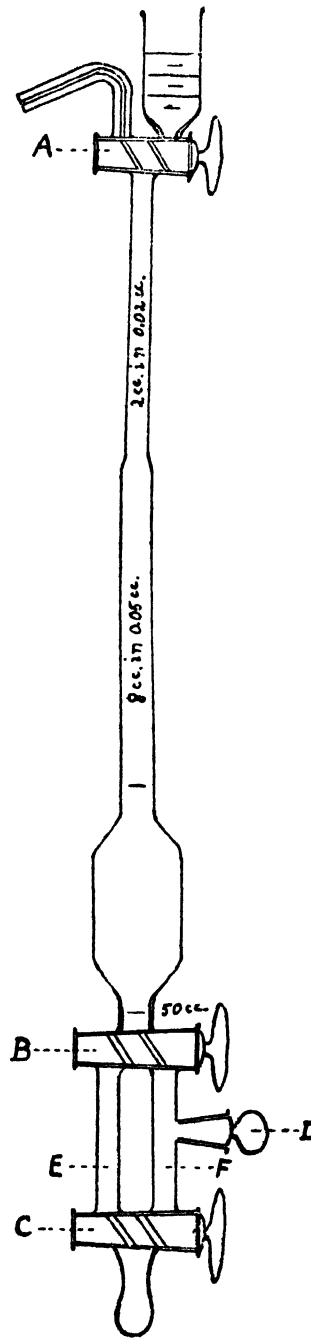


FIG. 2. APPARATUS FOR THE GASOMETRIC DETERMINATION OF CARBON DIOXIDE.
*(By courtesy of Soil Science.)*

The cock *B* is then turned through a complete revolution establishing, as connection is made momentarily between them, equilibrium in gas pressure in the tube *F* and the evacuated space above it. The leveling bulb is then raised to a position above *A* and this cock opened, allowing the escape of the entrapped air. Repeating this operation several times reduces the air in the apparatus to a negligible amount. The sample is now held in a gas-free apparatus.

Approximately N hydrochloric acid is next poured into the cup above *A* and exactly 2.5 cc. admitted to the buret, the leveling bulb being held about even with the stopcock *B*. The bulb is then lowered to the lowest position and the mercury allowed to flow out through the tube *E*, the cock *B* being closed while a little mercury still remains above it. If the sample is of such material that it is still contained in *D*, the mercury in the tube below it is now permitted to flow out through *C*, which is closed, leaving a few drops above this cock to seal it. *B* is then turned to allow the acid to run into the tube *F*.

The sample, if not originally weighed into the tube, is shaken out of the stopper into the acid. No precautions need be taken to moderate the violence of the reaction as any particles of the sample carried up into the bulb will be decomposed later. The apparatus should be shaken so that all of the sample is washed out of the stopper *D* and down from the walls of the tube.

When the evolution of gas has stopped, the communication between the leveling bulb and *F* is opened and the stopper and tube completely filled with mercury up to the 50 cc. mark. *B* is then closed and the apparatus shaken with a rotary motion in such a way that the liquid is distributed in a thin layer about the walls of the bulb until equilibrium between the gas in solution and that in the free space is attained. The liquid is next quickly drawn back into *F*, by lowering the leveling bulb and opening *B* which, however, is closed before any gas passes into it. *C* and *B* are finally turned to allow mercury to flow up into the buret through *E* while the acid is retained in *F*, the leveling bulb is raised until the mercury surface in it is on a level with that in the buret and the gas volume read. A fraction of a cc. of acid will unavoidably be held in the buret. This will cause no appreciable error in the results but care must be taken to read the gas volume at the surface of this liquid and not at the mercury surface, although it is the levels of the two mercury surfaces that are equalized.

The temperature and barometer readings should be noted at the time of reading the gas volume which affords sufficient data to permit the calculation of the weight of carbon dioxide obtained from the sample by means of tables[1]. For a complete discussion of the principle of the method the reader is referred to the original article. Suffice it to say that it depends upon the generation of gas in a Torricellian vacuum, the measurement of that portion of the gas contained in a volume of 47.5 cc. in equilibrium with the gas dissolved in 2.5 cc. of water and the calculation of the total volume of gas from its known solubility in water at the temperature of the determination, correction being made for the air dissolved in the 2.5 cc. of water. (This may, however, be determined for each analysis by introducing a few drops of alkali into the apparatus through the cup after reading the total volume of gas. The carbon dioxide will, of course, be absorbed leaving the air, the volume of which may then be read off after equalizing the mercury levels.)

Table 1 gives the results of several determinations by the two methods on a sample of calcite and three brands of baking powder. It shows the usual magnitude of experimental error to be expected in each as well as the agreement between the two methods.

[1] *J. Biol. Chem.*, 1917, **30**: 317, 360.

TABLE 1.

*Comparison of absorption and gasometric methods for determining carbon dioxide.*

| SAMPLE | CARBON DIOXIDE | | SAMPLE | CARBON DIOXIDE | |
|---|---|---|---|---|---|
| | Absorption | Gasometric | | Absorption | Gasometric |
| | *per cent* | *per cent* | | *per cent* | *per cent* |
| Calcium Carbonate. | 43.95 | 43.92 | Baking Powder, Sample No. 2.. | 13.41 | 13.39 |
| | 43.85 | 43.95 | | 13.36 | 13.28 |
| | 44.02 | 43.88 | | ..... | 13.39 |
| Baking Powder, Sample No. 1... | 13.15 | 13.16 | Baking Powder, Sample No. 3.. | 16.91 | 16.97 |
| | 12.97 | 13.18 | | 17.00 | 16.89 |
| | ..... | 13.23 | | .... | 16.92 |

## CONCLUSIONS.

It has been found impossible to get accurate results with the Knorr method, as prescribed in the official methods. This is due to two causes—the high concentration of acid designated and the time specified for expelling the liberated carbon dioxide. Hydrochloric acid was invariably carried over into the absorption vessels yielding high results unless the acid was considerably diluted. Aspiration continued only during the period of cooling is entirely insufficient to completely expel all of the carbon dioxide into the absorption train.

Following Heidenhain's technique, excellent results may be obtained with either his or Knorr's form of apparatus.

Equally good results can be obtained with the gasometric method described, page 188, which requires but a small fraction of the time necessary to carry out a determination by the absorption method.

In the light of the above facts, it seems desirable to revise the official methods of this association.

No report on soft drinks was made by the referee.

# REPORT ON EGGS AND EGG PRODUCTS.

By H. L. LOURIE (U. S. Food and Drug Inspection Station, U. S. Appraiser's Stores, New York, N. Y.), *Referee.*

In 1919 it was recommended that a further study be made of the methods for the determination of lecithin-phosphoric acid in dried eggs and alimentary pastes. A preliminary investigation was started by the referee and M. G. Wolf, of the New York Food and Drug Inspection Station, to determine the accuracy of the present method, commonly known as the Juckenack method, and to devise, if possible, a method which would give a greater recovery of the lecithin-phosphoric acid.

In conversation with the referee, C. L. Alsberg suggested that the phosphatids of wheat may be more soluble in methyl alcohol than ethyl alcohol. The work here reported is based largely on his suggestion.

This investigation has only reached the preliminary stage, but the writer is able to report that ethyl alcohol does not dissolve all of the phosphatids of flour or egg. Thirty grams of flour, which had been dried for 6 hours in a vacuum oven at a temperature of 50°C., vacuum 28½ inches, were extracted for 10 hours with absolute methyl alcohol and absolute ethyl alcohol. It was found that the amount of lecithin-phosphoric acid recovered in the case of the methyl alcohol was 0.028 and 0.025 per cent and, in the case of the ethyl alcohol, 0.016 and 0.014 per cent.

Another series of determinations was made in the same way and nitrogen was determined on the extracted matter. In the case of the methyl alcohol 0.067 per cent of nitrogen was obtained and, in the case of ethyl alcohol, 0.033 and 0.028 per cent.

A mixture was made of 29.4 grams of flour with 0.06 gram of whole egg. This material previous to mixing had been desiccated for 6 hours at 50°C., vacuum of 28½ inches. After extraction for 10 hours the lecithin-phosphoric acid was determined and, in the case of the methyl alcohol, gave 0.043 and 0.045 per cent and, in the case of ethyl alcohol, 0.024 and 0.023 per cent, making the proper correction for the blanks obtained in both the methyl and ethyl alcoholic extractions. This 2 per cent egg mixture would yield 0.0175 per cent of phosphoric acid in the case of methyl alcohol as compared with 0.0085 per cent of phosphoric acid for ethyl alcohol.

A modification by H. B. Mead of the method used at the New York Food and Drug Inspection Station for the determination of zinc in dried egg products, which has been used for the past year and a half in routine regulatory work and has given the utmost satisfaction, is as follows:

*Modified Method for Determination of Zinc in Egg Products.*

Place 25 grams of the well-mixed sample in an 800 cc. Kjeldahl flask; add 5 grams of zinc-free potassium sulfate, 3-4 glass beads to prevent bumping, 30 cc. of concentrated sulfuric acid, in the case of yolks or whole eggs (25 cc. of the acid in the case of albumins); and 30 cc. of concentrated nitric acid. Do not heat. When spontaneous action subsides, add 10 cc. of concentrated nitric acid. After two or three additions of concentrated nitric acid the action becomes less violent. Heat gently, at first, continuing the addition of concentrated nitric acid and increasing the temperature as the digestion proceeds until the contents of the flask is straw colored or colorless after nitric acid fumes have been boiled off. This digestion may be accomplished in the case of albumin in 40 minutes and in the case of yolks or whole eggs in 1 hour. To the warm liquid add 100 cc. of water; pour into a 400 cc. beaker and rinse the flask with two successive 50 cc. portions of water. To the combined water solution add concentrated ammonium hydroxide until faintly alkaline. Pass hydrogen sulfide gas through the solution for 15 minutes which should be sufficient to saturate. (At this

point the majority of albumins indicate the presence or absence of zinc. In the case of albumin, if zinc is present, add 1 cc. of a diluted solution of ferric chloride containing 0.5 gram of solid ferric chloride per 100 cc. This will assist in retaining zinc sulfide on the paper when filtering. Pass hydrogen sulfide gas through the solution for 15 minutes.) Heat the beaker on a steam bath for 30 minutes; remove; and allow to settle for 5–10 minutes. Then decant through a 9 cm. filter paper, allowing as much of the precipitate as possible to drain thoroughly. Dissolve the zinc sulfide from this precipitate with 10% hydrochloric acid, the solution after passing through the filter paper being returned to the original beaker. Copper and lead sulfides are insoluble at this point, and may be determined by the usual methods. To the hydrochloric acid solution add 5 grams of ammonium chloride, an excess of bromine water and a slight excess of concentrated ammonium hydroxide. Neutralize carefully with 10% hydrochloric acid adding 2 cc. in excess; add 10 cc. of 50% by weight of ammonium acetate and 8–10 drops of 10% ferric chloride solution, or enough to give a distinct reddish tinge. Dilute to about 300 cc. with water and boil for 1 minute. Allow to settle, filter while hot and wash with hot 5% ammonium acetate. Pass hydrogen sulfide gas through the filtrate for 15 minutes. Heat for 30 minutes on a steam bath; filter through a weighed, heavily padded Gooch crucible, using gentle suction. Wash with hot 5% ammonium acetate solution. Dry in oven; then ignite, roasting first. The increased weight of the Gooch crucible is due to oxide of zinc. This, multiplied by 0.8034, gives the amount of zinc present in a 25-gram sample.

An idea of the accuracy of this method may be gained by the following work performed by H. B. Mead. A sample of albumin was analyzed and found to have a blank of 1.3 mg. of zinc oxide.

The following determinations were made after adding definite amounts of zinc oxide:

TABLE 1.

*Determination of zinc oxide in albumin.*

| ZINC OXIDE ADDED | ZINC FOUND (AS ZINC OXIDE) AFTER SUBTRACTION OF BLANK | AVERAGE |
|---|---|---|
| *gram* | *gram* | *gram* |
| 0.0019 | 0.0032<br>0.0025 | 0.0028 |
| 0.0040 | 0.0052<br>0.0042 | 0.0047 |
| 0.0205 | 0.0206<br>0.0198 | 0.0202 |
| 0.0410 | 0.0416<br>0.0408 | 0.0412 |

This method is an improvement over the one originally used at the New York Food and Drug Inspection Station because it allows the rapid handling of a large number of samples. For example, it is comparatively easy to run at least 12 determinations for zinc within 24 hours, whereas with the old method at least 3 days were necessary.

Since the campaign inaugurated by the Bureau of Chemistry to prevent the importation of dried eggs containing large amounts of zinc, such a revolution has been caused in the methods used in China for the manufacture of this product that there is practically no dried egg product being offered for entry in this country which contains excessive amounts of zinc, the Chinese now largely using aluminium plates for the drying of the eggs.

*McGeorge Method for Determination of Zinc in Dried Egg Products.*

Weigh 25 grams of the sample into an 11 cm. silica dish. Add 5–10 cc. of olive or other vegetable oil and 5 cc. of a saturated solution of sodium carbonate, cover with two 9 cm. filter papers and heat over an asbestos gauze to preliminary ashing. Transfer to a muffle furnace and heat at low redness until the ash is white or nearly so. (Albumin is likely to swell badly in the muffle, according to the stage to which the preliminary ashing is carried.)

Cool the dish and contents, add 50 cc. of water, 10 cc. of concentrated ammonium hydroxide and 10 cc. of a saturated solution of ammonium carbonate. Heat to boiling and filter. (In cases where an excessive amount of zinc is present it is necessary to heat the residue insoluble in ammonium hydroxide and treat again as above to dissolve all of the zinc.) Acidify the combined filtrates with acetic acid, boil to remove the excess carbon dioxide and pass hydrogen sulfide through to complete precipitation of zinc. Filter on a tared Gooch crucible, ignite and weigh as zinc oxide.

The method devised by W. T. McGeorge for the determination of zinc in dried egg products has been found to be very accurate by the San Francisco Food and Drug Inspection Station. McGeorge obtained the following results using the association method and the McGeorge method:

TABLE 2.

*Comparison of zinc determinations in dried egg products.*

| DESCRIPTION OF SAMPLE | MC GEORGE METHOD | ASSOCIATION* METHOD |
|---|---|---|
| | *gram* | *gram* |
| Egg yolk (25 grams) containing no added zinc.. | 0.0031 | 0.0034 |
| Same egg yolk after the addition of known weights of zinc oxide before ashing..... | 0.0238†<br>0.0298‡ | .....<br>..... |
| Sample of egg albumin of high zinc content refused entry at San Francisco..... | 0.0585 | 0.0694 |

**Assoc. Official Agr. Chemists, Methods,* 2nd ed., 1920, 151 (except that sample was ashed at dull redness and taken up in hydrochloric acid instead of destroying by acid digestion).

† Zinc oxide added, 0.0203 gram; present, 0.0031 gram; total present, 0.0234 gram.

‡ Zinc oxide added, 0.0269 gram; present, 0.0031 gram; total present, 0.0300 gram

All of the above determinations were made on 25-gram portions.

## RECOMMENDATIONS.

It is recommended that next year corroborative studies be made of the McGeorge method.

The referee was instructed to study the methods for the detection of decomposition in dried eggs. It is recommended that no time be spent in studying such methods unless such work can be performed at the place where the dried eggs are actually manufactured. It is obvious that any results obtained on dried eggs which had been shipped from China to this country would be useless unless they could be corrolated with results obtained on the eggs during the period of manufacture and under manufacturing conditions. It is a well-known fact that it is a common occurrence at Chinese factories to add ammonia to the eggs while they are being dried. Previous to the change in the process of manufacture which has been caused by the Bureau of Chemistry action against egg products high in zinc, it was also a common practice to add zinc chloride as a preservative while the eggs were being dried.

Committee C requested your referee to prepare a set of methods covering the usual determinations made in the analysis of eggs and egg products. Since the meeting of the association in 1919, a bulletin has been published[1] which gives the results of a large number of determinations of eggs of various compositions, analyzed at various sections of the country under varying laboratory conditions. The results, in general, show that whether eggs are examined in Washington, Philadelphia, New York, Chicago or San Francisco, the analytical results agree very closely, and that, when carefully followed, the analytical methods described in that bulletin will give concordant results in the hands of a number of analysts. The bulletin gives a full description of the methods used, as well as the directions, and it is recommended that the referee for the coming year present to the association a tentative set of methods for the analysis of eggs and egg products, based on those used in this report.

It is recommended that a study be made to determine the preservative best suited to be used in frozen eggs which are offered for analysis in routine regulatory work. It has been the experience at the New York Food and Drug Inspection Station that frozen eggs which have been melted undergo very rapid fermentation. A suitable preservative, which could be added immediately to the samples, would make the handling of this material much easier for the analyst and would insure more accurate results. Toluol has been used as a preservative at the writer's laboratory with very unsatisfactory results, as it has been found that a sample of frozen eggs preserved with a layer of toluol decomposes very rapidly. It is, of course, impossible to use formaldehyde because of the formation of compounds with ammonia. It is believed that chloroform or sodium fluoride will make the best preservative for material of this type.

No report on food preservatives was made by the referee.

[1] U S. Dept. Agr. Bull. 846: (1920).

# REPORT ON COLORING MATTERS IN FOODS[1].

By W. E. MATHEWSON (Bureau of Chemistry, Washington, D. C.), *Referee.*

No methods for the analysis of commercial food colors have yet been considered by the association. While food colors are used in relatively small quantities, they are particularly likely to contain objectionable impurities because of the methods used for their manufacture and the lack of knowledge concerning them on the part of the public—a fact that led to the establishment of the certification procedure by the Department of Agriculture some twelve years ago. The following report has been prepared to bring this matter before the association and to provide a draft to aid in the selection of a set of official methods for the analysis of such products.

The methods given in this report are for the most part those used or developed in the New York Food and Drug Inspection Station in 1908 to 1910[2]. These methods have been slightly modified or elaborated in many cases but when such changes give a distinctive form to the procedure the original method has also been described. A few statements and tests in the bulletin have been omitted.

The methods for the estimation of arsenic were later much improved by C. R. Smith by the use of potassium iodide and stannous chloride for the reduction of pentavalent arsenic and the sensitization of the zinc. It is understood that the arsenic method with these improvements is being recommended for adoption by the association so it is not described in this report.

All methods for the estimation of arsenic after treatment with nitric acid have been written so that troublesome filtrations are partly avoided by the use of aliquot portions.

Among volumetric reduction methods for the direct estimation of coal tar dyes that of Knecht and Hibbert, depending upon the use of titanium trichloride, has probably been given the most thorough study by analysts. It has been used by the referee for many years with the food colors and considered very useful. The monograph by Knecht and Hibbert although showing the method to be a general one does not describe experiments with any of the food colors except Naphthol Yellow S; and the concentrations of the standard solutions they employ are about 0.3 to 0.4 N. Solutions 0.100 to 0.103 N are much more convenient in routine work and for these reasons it has been thought best to give a rather complete description of the method, presenting it

[1] Abstract.

[2] U. S. Bur. Chem. Bull. 147: (1912). Other publications that have been of special value in preparing the description of the methods are: Allen's Commercial Organic Analysis. 4th ed., 1911, 5; Ludwig Gattermann. The Practical Methods of Organic Chemistry. Translated by W. B. Schober and V. B. Babasinian. 3rd American from the 11th German ed., 1916; and A. E. Leach. Food Inspection and Analysis. Revised and Enlarged by A. L. Winton. 4th ed., 1920.

in the form considered most suitable for food-color analysis. Not the least of the practical advantages of the titanium trichloride method is its applicability to the rapid determination of iron, copper, and other common substances.

The results obtained by different analysts in determining dyes by colorimetric comparison seem to vary a good deal in reliability and accuracy. On this account the methods have been placed under three headings so that those involving the use of a specially suitable light might be more readily specified.

Ordinary colorimetric measurements in white light are particularly unsatisfactory with yellow solutions which ordinarily show marked absorption only in the deep blue and violet regions of the spectrum. Small differences existing between the absorptive power of two such solutions are difficult to perceive because the emergent residual violet light is diluted with the red, green, yellow and light blue rays of much greater visibility which are transmitted without loss by both solutions. If the solutions are rather concentrated the comparison becomes still more uncertain for, in this case, the absorption is nearly complete in the violet and such difference as may be observed comes essentially from an extremely narrow region at the edge of the absorption band. This difference may be quite obscured by the presence of a trace of a more reddish coloring matter so that colorimetric comparison of such concentrated solutions gives results of no practical value. Obviously, difficulties of this sort are largely avoided if the comparison is made in violet light. The mercury arc with screens selected to absorb all of the strong radiation except that of wave length $0.436\mu$ gives a light particularly suitable for this work.

Spectrophotometric methods might be described or discussed more fully but many types of apparatus are in use, each requiring its own form of procedure. The spectrophotometer because of its convenience and wide applicability can scarcely fail to come into more general use in analysis. A new form of the instrument of simple construction has recently been devised by I. G. Priest[1] for use with dye solutions, oils and similiar liquids. The transmissive indices (extinction coefficients) as well as the transmissions are read directly without computation. A special advantage of the design lies in the fact that the illumination of the field can be adjusted directly to the intensity best suited to the eye so that all of the (homogeneous) light may be utilized.

For accurate colorimetric and spectrophotometric work, sources of strong monochromatic light are of great importance. The mercury arc with suitable screens gives fairly homogeneous radiation of the respective wave lengths $0.436\mu$, $0.546\mu$ and $0.577$–$0.579\mu$.

[1] *Cotton Oil Press*, July, 1920, 4: 96.

The methods have been given in the following order:

Moisture.
Total matter insoluble in water.
Inorganic or non-volatile matter insoluble in water.
Total matter soluble in water.
Matter insoluble in carbon tetrachloride.
Sodium chloride.
Sodium sulfate.
Sulfated ash.
Heavy metals.
Calcium.
Arsenic by direct precipitation.
Arsenic after treatment with nitric acid.
Total arsenic.
Sulfur.
Nitrogen.
Total halogens.
Total iodine.
Sodium iodide.
Ether extractives.
Dye by titration with titanium trichloride.
Dye by titration with potassium permanganate.
Dye by colorimetric comparison.
Dye by spectrophotometer.
Lower sulfonated dyes.
Melting point.
Martius Yellow in Naphthol Yellow S.
Boiling point of Cumidine from Ponceau 3R.
Orange II in Orange I.
Iodeosine G in Erythrosine.
Isomeric and similar dyes in Amaranth.
Sodium chloride in dyes or mixtures containing more than 25 per cent of this substance.
Sucrose in dye mixtures.
Sodium carbonate in Erythrosine.

## METHODS FOR THE ANALYSIS OF COAL TAR FOOD COLORS.

### MOISTURE.

(a) (Applicable with Ponceau 3R, Orange I, Tartrazine, Amaranth, Light Green S. F. Yellowish, Erythrosine and Indigo Disulfoacid.)

Heat 2 grams of the finely ground dye at 135°C. in a current of hydrogen until constant weight is attained.

(b) (Applicable with Naphthol Yellow S, Ponceau 3R, Orange I, Tartrazine, Amaranth, Light Green S. F. Yellowish, Erythrosine and Indigo Disulfoacid.)

Dry 2 grams of the finely ground dye at 135°C. in an air oven to constant weight. The moisture is usually driven off in from 2–4 hours.

(c) (Applicable with Yellow A. B. and Yellow O. B.)

Dry 2 grams of the finely ground dye at room temperature in a desiccator containing sulfuric acid until constant weight is attained.

(d) (Applicable with Yellow A. B. and Yellow O. B.)

Dry 2 grams of the powdered dye in an air oven or vacuum oven at 80°C. to constant weight.

### TOTAL MATTER INSOLUBLE IN WATER.

(a) (Applicable with Naphthol Yellow S, Tartrazine, Amaranth, Light Green S. F. Yellowish and Erythrosine.)

Dissolve 5 grams of dye in 200 cc. of hot water, filter on a Gooch crucible, wash until the washings are colorless, dry at 100–105°C. and weigh.

(b) (Applicable with Ponceau 3R, Orange I, and Indigo Disulfoacid.)

Stir 5 grams of the dye with 250 cc. of hot water, heat the mixture to boiling and boil for 3 minutes with occasional stirring. Filter on a tared 25 cc. Gooch crucible, wash, dry and weigh as stated under (a). With low-grade dyes the filter sometimes becomes clogged. In such a case the determination must be repeated, using a smaller charge, but when this is done the weight of the charge taken must always be stated.

### INORGANIC OR NON-VOLATILE MATTER INSOLUBLE IN WATER.

(a) (Applicable with Naphthol Yellow S, Ponceau 3R, Orange I, Tartrazine, Amaranth, Erythrosine, Light Green S. F. Yellowish and Indigo Disulfoacid.)

Ignite the Gooch crucible containing the total insoluble matter at a low red heat until organic matter is incinerated completely, cool and weigh.

### TOTAL MATTER SOLUBLE IN WATER.

(a) (Applicable with Yellow A. B. and Yellow O. B.)

Transfer 5 grams of the well-powdered dye to a 500 cc. Erlenmeyer flask or wide-mouthed bottle, add 200 cc. of water, stopper and mix thoroughly by shaking. Allow to stand 2 hours with occasional shaking, filter, and evaporate 100 cc. of the filtrate in a tared platinum dish. Dry at 100–105°C. and weigh. Test small portions of the filtrate for chlorides, sulfates, nitrates, etc. If more than traces of these are present, determine them in aliquot portions of the filtrate by the usual methods.

### MATTER INSOLUBLE IN CARBON TETRACHLORIDE.

(a) (Applicable with Yellow A. B. and Yellow O. B.)

Mix 5 grams of the dye in a 100 cc. beaker with 50 cc. of carbon tetrachloride, stir and heat to boiling. Filter the hot solution on a tared Gooch crucible, transfer the residue in the beaker to the filter and complete the washing with an additional 50 cc. of carbon tetrachloride used in portions of 5–10 cc. each. Dry at 100–105°C. and weigh.

### SODIUM CHLORIDE.

(a) (Applicable with Ponceau 3R, Orange I, Tartrazine, Amaranth, Light Green S. F. Yellowish, and Indigo Disulfoacid.)

Mix 5 grams of dye thoroughly with 4–6 grams of potassium carbonate or sodium carbonate in a 50 cc. platinum dish, moisten with water or 50% alcohol, cover evenly with about 1 gram of the powdered carbonate, dry, and ignite at a low red heat until organic matter is destroyed. Allow to cool, add enough water to form a thin paste and if the mass does not disintegrate as the soluble salts dissolve, break up the lumps with a glass rod. The charred mass should soften under the action of the water so that very little stirring is necessary to produce a uniform suspension. Wash or transfer the mixture into a 250 cc. graduated flask with 100–150 cc. of hot water and add an excess of potassium permanganate to oxidize sulfides. Destroy the excess of permanganate by adding sulfur dioxide solution until the red color changes to brown, then cool the mixture and make up to the mark with water. Filter through a dry paper, acidify 100 cc. of the filtrate with nitric acid and precipitate the chlorine by adding a slight excess of silver nitrate. Heat with stirring until the silver chloride has coagulated, cool, then filter on a tared Gooch crucible and finally dry and weigh the silver chloride in the usual manner. If the solution should be brown and the silver chloride tend to pass through the filter the charge has not been completely charred and the determination must be repeated.

(b) (Applicable with Ponceau 3R, Tartrazine, Orange I, Amaranth, Light Green S. F. Yellowish and Indigo Disulfoacid.)

Proceed exactly as described in (a) to the point at which the suspension of the disintegrated charred mass is obtained in a 250 cc. graduated flask, allow to stand until all soluble salts are dissolved, cool, dilute to the mark with water, mix thoroughly and filter through a dry paper. Measure an aliquot portion of 200 cc. of the filtrate into a 600 cc. beaker and add a sufficient amount of 6–7% solution of potassium permanganate to oxidize the sulfides and produce a permanent pink color. Then add about

50 cc. of water and a slight excess of silver nitrate solution. Six to eight cc. of 10% silver nitrate solution are usually sufficient to precipitate the chlorine. Partially cover the beaker with a watch glass and acidify by carefully adding about 12 cc. of concentrated nitric acid (sp. gr. 1.42). Heat nearly to boiling, then add 1–2% sulfur dioxide solution slowly, with stirring, until the oxides of manganese dissolve leaving the white silver chloride. Boil until any excess of sulfur dioxide is removed, cool and finally filter, wash and weigh the silver chloride in the usual manner.

(c) (Applicable with Naphthol Yellow S.)

Dissolve 5 grams of dye in 250 cc. of water, filter if necessary, add 5 cc. of concentrated nitric acid and precipitate the chlorine by adding a slight exces of silver nitrate. The silver chloride is separated, washed, ignited and weighed on a tared Gooch crucible in the usual way. The determination may be made conveniently in connection with that of insoluble matter.

(d) (Applicable with Ponceau 3R.)

Dissolve 5 grams of the dye in 150 cc. of hot water, wash into a 250 cc. graduated flask and add 25 cc. of a 10% solution of barium nitrate. Cool the mixture, make up to the mark, mix, and filter through a dry paper; acidify 100 cc. of the filtrate, representing 2 grams of color, with nitric acid and treat with a slight excess of silver nitrate. The precipitated silver chloride is separated, washed, ignited and weighed in a tared Gooch crucible in the usual way.

(e) (Applicable with Erythrosine.)

Dissolve 5 grams of the dye in 400 cc. of water and add a mixture of 2 cc. of concentrated nitric acid with 10–20 cc. of water, then dilute the mixture to 500 cc., mix, and filter through a dry filter. Reserve an aliquot portion of the filtrate for the estimation of sulfates. Treat 200 cc. of the filtrate with slightly more silver nitrate solution than is required to precipitate the halogens present, add 5 cc. of concentrated nitric acid, heat to boiling, cool and filter the precipitate on a weighed Gooch crucible; dry, cool and weigh. Test a small portion of the filtrate from the color acid by adding a few drops of sulfuric acid, a drop of sodium nitrite solution, and a few drops of carbon tetrachloride or starch paste. If an appreciable amount of iodide is present it must be determined as stated under sodium iodide (a), and correction made by subtracting, from the weight of the precipitate, the weight of silver iodide obtained in the sodium iodide estimation. The difference is then calculated to sodium chloride.

### SODIUM SULFATE.

(a) (Applicable with Light Green S. F. Yellowish.)

Dissolve 2 grams of dye in 50 cc. of hot water, filter on a small paper and wash the residue and filter with hot water. Dilute the combined filtrate and washings to about 200 cc., add ½ cc. of concentrated hydrochloric acid, heat to boiling and add a slight excess of 10% barium chloride solution. Allow the mixture to stand overnight, filter on a weighed Gooch crucible, wash, ignite and weigh in the usual manner.

(b) (Applicable with Erythrosine.)

Employ an aliquot portion of not less than 100 cc. of the filtrate obtained after precipitating the color acid as described under the determination of sodium chloride, (e). Precipitate as barium sulfate, making the determination in the usual way.

(c) (Applicable with Amaranth and Tartrazine.)

Introduce 5 grams of the dye into a 250 cc. graduated, stoppered flask and dissolve in 200 cc. of warm water. Add 70 grams of pure pulverized sodium chloride, stopper the flask and shake or stir the mixture gently at frequent intervals for 1 hour. The salt will dissolve and ordinarily the appearance of the mixture will show that the dye has been almost completely precipitated. In warm weather or with impure dyes the super-saturated solution first formed will be more stable and it may be necessary to

cool the mixture by placing the flask in cold water. Dilute the mixture containing the precipitated dye to 250 cc. with a saturated solution of sodium chloride, mix, and filter on a dry, 18 cm. filter paper. Dilute 100 cc. of the filtrate with 200 cc. of water, add 0.1 cc. of concentrated hydrochloric acid, heat to boiling and precipitate the sulfates with slight excess of barium chloride solution. Allow the mixture to stand for several hours or overnight, filter off the barium sulfate on a tared Gooch crucible, wash, ignite and weigh in the usual manner.

(d) (Applicable with Amaranth and Tartrazine.)

Dissolve 2 grams of dye in 100 cc. of warm water in a 200 cc. graduated flask and add 36 grams of pure sodium chloride. Allow the mixture to stand with frequent shaking for 1 hour and after cooling make up to the mark with a saturated salt solution. Shake the mixture, then filter through a dry paper; dilute 100 cc. of the filtrate (representing 1 gram) with water, acidify with 0.1 cc. of concentrated hydrochloric acid and precipitate the sulfates with barium chloride. The precipitate is separated, washed and ignited on a tared Gooch crucible.

(e) (Applicable for Ponceau 3R, Orange I and Indigo Disulfoacid.)

Proceed as described under (c) using, however, a 50-gram portion of pulverized sodium chloride instead of the 70-gram portion.

(f) (Applicable with Naphthol Yellow S.)

Dissolve 2½ grams of dye with about 300 cc. of water in a 500 cc. graduated flask, add 100 cc. of a 20% solution of potassium chloride, shake the mixture well and dilute to 500 cc. with water. Shake again, then filter through a dry paper. Treat an aliquot portion of the filtrate, representing not less than 1 gram of the dye, with 5 cc. of 10% barium chloride solution and allow to stand overnight. If a precipitate forms it is washed, dried and weighed in the usual manner.

### SULFATED ASH.

(a) (Applicable with Naphthol Yellow S, Ponceau 3R, Orange I, Tartrazine, Amaranth, Light Green S. F. Yellowish, Erythrosine, and Indigo Disulfoacid.)

Weigh accurately 2 grams of dye in a tared platinum basin of about 100 cc. capacity, moisten with a little dilute sulfuric acid (15-20%) and rotate the dish to spread the pasty mixture over the bottom. Warm over a ring burner or similar device, gently at first to avoid spattering, finally at a somewhat higher temperature to carbonize the mass and volatilize the sulfuric acid. Moisten the residue with concentrated sulfuric acid and ignite, beginning at a low temperature and gradually increasing the heat to low redness; repeat the treatment with acid and ignition until all carbonaceous matter is removed and a white or reddish ash is obtained. Heat this cautiously over the blast lamp until it fuses to a clear, limpid liquid and the effervescence, due to the decomposition of the sodium acid sulfate, has just ceased, then allow to cool in a desiccator and weigh.

(b) (Applicable with Yellow A. B. and Yellow O. B.)

Heat 2 grams of the dye in a tared platinum dish or crucible at a low temperature until almost all of the coloring matter has volatilized; moisten the residue with concentrated sulfuric acid and heat cautiously at first, then to low redness, to burn off all carbonaceous matter. If the residue incinerates with difficulty it will be necessary to repeat the treatment with sulfuric acid and subsequent ignition several times. When carbonaceous matter is completely destroyed, ignite over the blast lamp, cool and weigh.

### HEAVY METALS.

(a) (Applicable with Naphthol Yellow S, Ponceau 3R, Orange I, Tartrazine, Amaranth, Light Green S. F. Yellowish, Erythrosine, and Indigo Disulfoacid.)

Moisten the sulfated ash with a few cc. of concentrated hydrochloric acid and evaporate to dryness on the steam bath. Warm the residue with a mixture of 1 cc. of concentrated hydrochloric acid and about 20 cc. of water until all soluble material has been dissolved; transfer to a 100 cc. graduated flask or cylinder, dilute with water to 100 cc., mix and pour on a dry filter. Reserve 50 cc. of the filtrate for the estimation of calcium. Place 10 cc. of the filtrate in a test tube, add 10 cc. of freshly prepared hydrogen sulfide test solution (U. S. P.), shake the mixture, warm to 50°C., stopper and allow to stand in a warm place (about 35°C.) for 30 minutes. Run a blank test at the same time with the same amount of hydrogen sulfide solution, using water instead of the solution containing the ash. No turbidity other than that produced by a slight separation of sulfur should appear in this test. Both tubes are then made slightly alkaline with ammonium hydroxide and no precipitate should be produced, although a slight coloration, due to the presence of a small amount of iron, sometimes occurs. If this coloration is very marked, the amount of iron should be determined. This is done by digesting the sulfated ash from a weighed amount of the sample with hydrochloric acid until all the iron has gone into solution. Filter the liquid and pour the filtrate into an excess of hot, pure, freshly prepared sodium hydroxide solution in a platinum dish. Wash the precipitate, dissolve in dilute hydrochloric acid and again precipitate with ammonium hydroxide. Wash the last precipitate, ignite and weigh in the usual manner.

(b) (Applicable with Yellow A. B. and Yellow O. B.)

If the residue from the sulfated ash consists mainly of iron oxide, silica, or similar insoluble compounds, fuse it in the dish with about 1 gram of potassium carbonate or sodium carbonate until any silicates have been decomposed. Moisten the residue with 2–3 cc. of concentrated hydrochloric acid, evaporate to dryness on the steam bath and continue as directed under (a). The preliminary fusion with alkaline carbonate may be omitted if the ash consists chiefly of sodium sulfate.

### CALCIUM.

(a) (Applicable with Naphthol Yellow S, Ponceau 3R, Orange I, Tartrazine, Amaranth, Light Green S. F. Yellowish, Erythrosine, Indigo Disulfoacid, Yellow A. B. and Yellow O. B.)

The aliquot portion of the solution reserved for the estimation of the calcium, as directed under the determination of heavy metals, (a), represents the sulfated ash from 1 gram of dye. Heat the solution to boiling, add a slight excess of ammonium hydroxide solution, boil a few minutes and filter off any ferric hydroxide that may be precipitated, on a small paper filter. Wash the filter paper and residue with a little water, collecting the washings in the same beaker with the filtrate. Heat the solution to boiling, precipitate the calcium with a slight excess of ammonium oxalate and filter on a tared Gooch crucible. Wash the precipitate, dry at 100°C. and weigh as $CaC_2O_4.H_2O$. If preferred, the precipitate may be filtered on a Gooch crucible not previously tared, the asbestos and calcium oxalate rinsed into a beaker with about 100 cc. of water, 2 cc. of concentrated sulfuric acid added and the solution warmed and titrated against 0.1 N potassium permanganate solution in the usual manner.

### ARSENIC BY DIRECT PRECIPITATION.

(a) (Applicable with Naphthol Yellow S, Tartrazine, Amaranth, and Light Green S. F. Yellowish.)

Dissolve 10 grams of the dye in 250 cc. of water and add 10 cc. of strong bromine water. Make the mixture alkaline with 1–2 cc. of concentrated ammonium hydroxide solution, then add 20 cc. of a sodium phosphate solution containing 100 grams of the crystallized salt per liter. Finally add a slight excess of magnesia mixture (containing

55 grams of hydrated magnesium chloride, 55 grams of ammonium chloride and 88 cc. of ammonium hydroxide solution, sp. gr. 0.90, per liter). The amount of magnesia mixture used must be 1–5 cc. in excess of that required to completely precipitate the phosphate, as ascertained previously by experiment, and it must be poured into the dye mixture slowly, the latter being well stirred during the addition. Add 10 cc. of ammonium hydroxide (sp. gr. 0.90) and allow the mixture to stand for at least 30 minutes. Filter on an 18 cm. paper filter and wash with water containing one-tenth its volume of concentrated ammonia solution until practically all of the dye is removed. Wash with about 5 cc. of water, allow the filter containing the washed precipitate to drain 15–30 minutes to remove most of the adhering liquid. Finally dissolve the magnesium ammonium phosphate and arsenate by pouring several small portions of 10% hydrochloric acid over the filter. Dilute the total filtrate to 40 cc. with 10% hydrochloric acid and determine arsenic[1], the hydrochloric acid solution being substituted for the sulfuric acid mixture[2]. The hydrochloric acid solution is treated with potassium iodide, this and subsequent operations being carried out exactly as specified[3]. It is well to make the standard stains by treating 250 cc. portions of water with known amounts of a standard sodium arsenate solution, precipitating the arsenic as stated for the dye solution and carrying out the further analytical operations in exactly the same way.

(b) (Applicable with Erythrosine.)

Dissolve 18 grams of dye in 425 cc. of water, add 5 cc. of strong bromine water and 20 cc. of 10% hydrochloric acid. Mix, filter and treat 250 cc. of the filtrate (corresponding to 10 grams of dye) with 5 cc. of concentrated ammonium hydroxide solution (or a sufficient amount to render it slightly alkaline). Precipitate and determine the arsenic by the addition of sodium phosphate and further operations as described under (a).

### ARSENIC AFTER TREATMENT WITH NITRIC ACID.

(a) (Applicable with Naphthol Yellow S, Ponceau 3R, Orange I, Tartrazine, Amaranth, Light Green S. F. Yellowish, Erythrosine, and Indigo Disulfoacid).

Place 12.5 grams of the powdered dye in a 600 cc. beaker of Pyrex or similar resistant glass, add 25 cc. of concentrated nitric acid and, in case the dye tends to form a clot or cake, stir the mixture thoroughly. Heat to boiling, keep at boiling temperature for about 5 minutes, then add 150–200 cc. of water, pour into a graduated 250 cc. cylinder and dilute the mixture to 250 cc. Mix by pouring back into the beaker, and allow to stand for a few minutes, then filter on an 18 cm. paper filter. Treat 200 cc. of the filtrate, corresponding to 10 grams of dye, with 25 cc. of strong ammonium hydroxide solution (or a sufficient measured amount to make the solution alkaline). Finally precipitate and determine the arsenic by the addition of sodium phosphate solution and further operations as described under the determination of Arsenic by Direct Precipitation, (a), correcting for any traces of arsenic in the reagents by blank tests or standard stains obtained with the same amounts of the reagents.

(b) (Applicable with Yellow A. B., and Yellow O. B.)

Mix thoroughly 15 grams of the powdered dye in a 400 cc. beaker with 150 cc. of 10% nitric acid, heat to boiling, allow to cool, neutralize with ammonium hydroxide and measure the liquid in a graduated cylinder. Filter and determine the arsenic in an aliquot portion of the filtrate corresponding to 10 grams of dye by precipitation with magnesia mixture and subsequent operations as described under Arsenic by Direct Precipitation, (a).

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[1] *Assoc. Official Agr. Chemists, Methods.* 2nd ed., 1920, 147.
[2] *Ibid.*, 148, 4.
[3] *Ibid.*, 149.

### TOTAL ARSENIC.

(a) (Applicable with Naphthol Yellow S, Ponceau 3R, Orange I, Tartrazine, Amaranth, Light Green S. F. Yellowish, Erythrosine, Indigo Disulfoacid, Yellow A. B. and Yellow O. B.)

Digest 10 grams of the dye with a mixture of measured amounts of concentrated sulfuric acid and nitric acid until all organic matter is destroyed. It is recommended that the dye be introduced into a tall 600 cc. beaker of refractory glass provided with a watch glass cover, or into a 600 cc. Kjeldahl flask. Treat with 15 cc. of concentrated sulfuric acid, then add 25 cc. of concentrated nitric acid and digest slowly under a hood until the nitric acid has been decomposed or volatilized and the mixture begins to turn dark. Add cautiously a few cc. of nitric acid to the hot mixture which will again become light yellow or orange and repeat the addition of small quantities of nitric acid at intervals until the solution no longer shows a tendency to darken on further heating. The nitric acid must be taken from a previously measured portion of about 50 cc. or be added otherwise in such a manner that the total quantity used will be known. Allow the completely digested mixture to cool and add 200 cc. of water, then treat with concentrated ammonium hydroxide solution from a graduated cylinder until the mixture becomes slightly alkaline (corresponding to an excess of 1–3 cc.) and note the total amount added. Determine the arsenic in the solution by precipitation with phosphate solution and magnesia mixture and subsequent operations as described under Arsenic by Direct Precipitation, (a). The comparatively large amounts of reagents necessary for the destruction of the organic matter will usually contain appreciable quantities of arsenic for which correction must be made by blank determination. Measure into a roughly tared 600 cc. beaker quantities of the sulfuric and nitric acids equal to those used in the digestion. Heat under the hood until the sulfuric acid begins to volatilize, then allow to cool and weigh the beaker again on scales accurate to 1 or 2 grams. Calculate the approximate amount of acid (as sulfuric acid) in the original digestion mixture, after complete destruction of the dye, from the number of cc. of concentrated ammonium hydroxide solution that was required to neutralize it, and evaporate the mixture of acids measured off for the blank until the quantity remaining is nearly the same as that of the original mixture after digestion. Allow to cool and carry out the subsequent procedure as with the residue obtained by destruction of the dye. If the amount of arsenic found in the blank determination exceeds 0.000005 gram of arsenious oxide (corresponding to 0.5 parts per million with a 10-gram charge of dye) the correction to be made will be quite inaccurate and purer reagents should be secured for the work.

### SULFUR.

(a) (Applicable with Naphthol Yellow S, Ponceau 3R, Orange I, Tartrazine, Amaranth, Light Green S. F. Yellowish, Erythrosine, and Indigo Disulfoacid.)

Determine upon 0.2–0.3 gram portions by the Carius method, using 3 cc. portions of fuming nitric acid and heating the sealed tubes to 300°C. for at least 8 hours.

### NITROGEN.

(a) (Applicable with Naphthol Yellow S, Ponceau 3R, Orange I, Tartrazine, Amaranth, Light Green S. F. Yellowish, Indigo Disulfoacid, Yellow A. B. and Yellow O. B.)

Use the method of Dumas.

(b) (Applicable with Light Green S. F. Yellowish, and Indigo Disulfoacid.)

Determine on 2-gram portions by Gunning's modification of the Kjeldahl process, using a little copper sulfate to assist the oxidation.

(c) (Applicable with Ponceau 3R, Orange I, Tartrazine and Amaranth.)

Treat 2 grams of the color with 25 cc. of a saturated solution of sulfur dioxide and 1 gram of zinc dust, and warm the mixture gently until it becomes colorless. This should take place in 2–3 minutes, but if it does not, add more sulfur dioxide solution, in small portions at a time, until the color is destroyed. Then add 30 cc. of concentrated sulfuric acid and 0.7 gram of mercuric oxide, or its equivalent of metallic mercury, and digest the mixture. Finally make alkaline and distil as usual in the Kjeldahl method.

### TOTAL HALOGENS.

(a) (Applicable with Erythrosine.)

Mix 0.5–1 gram of the dye with 4 grams of potassium carbonate, moisten to a paste, dry, cover with a layer of dry potassium carbonate and ignite at a low red heat. Allow to cool, moisten with a few drops of water and break up the charred mass thoroughly. Wash into a beaker with about 200 cc. of water, allow to digest for 15 minutes and filter. Wash the insoluble matter until the washings no longer react with silver nitrate; then acidify the filtrate and washings with nitric acid and precipitate the halogens with silver nitrate. Filter, wash, and weigh the insoluble silver salts on a tared Gooch crucible in the usual manner.

### TOTAL IODINE.

(a) (Applicable with Erythrosine.)

Place 0.3–0.4 gram of the dye in a porcelain casserole, dissolve in 5 cc. of a 10% sodium hydroxide solution, then add 35 cc. of a 7% solution of pure potassium permanganate. After mixing, partially cover the vessel with a watch crystal and add 10 cc. of nitric acid. Agitate the mixture, place on a steam bath and keep covered until spattering ceases, after which remove the watch glass and allow evaporation to proceed to dryness. Care should be taken to prevent access of reducing gases or vapors to the mixture. Treat the residue with 5 cc. of 7% potassium permanganate and 5 cc. of concentrated nitric acid and again evaporate to dryness. Then add about 50 cc. of water and 5 cc. of concentrated nitric acid to the residue, following this by 40 cc. of a saturated solution of sulfur dioxide, and allow the whole to stand with occasional stirring (breaking up the lumps with a glass rod) until the hydrated oxide of manganese has dissolved. Filter and wash the filter paper thoroughly with water, add an excess of silver nitrate to the combined filtrate and washings and boil until sulfur dioxide has been expelled and the silver iodide has flocculated. Separate, wash and weigh the precipitate in the usual manner.

The solution of the oxides of manganese often requires some time, as the hardened residue is not rapidly attacked by the sulfur dioxide solution. With dyes known to be free from more than traces of salt or of other chlorine or bromine compounds it is unnecessary to evaporate the acid digestion mixture to dryness; heat to boiling with stirring to prevent bumping, allow to cool somewhat, and treat with about 50 cc. of cold water. Then add strong sulfur dioxide, not too slowly and while the mixture is being well stirred, until the oxides of manganese dissolve with the formation of a clear colorless solution. Treat the solution with a sufficient amount of silver nitrate solution to precipitate all of the iodine, heat to boiling and boil until sulfur dioxide has been expelled. Separate the silver iodide and weigh in the usual manner.

(b) (Applicable with Erythrosine.)

Mix 0.2–0.3 gram of the sample with 2 grams of pure potassium dichromate and 15 cc. of strong sulfuric acid in the evolution flask of an apparatus made entirely of glass with ground-glass joints. Thoroughly mix the contents of the evolution flask so that all lumps are disintegrated, heat at 100°C. for 15 minutes, after which raise the temperature to 150°C. for 30 minutes, a current of air, dried over calcium chloride and potassium hydroxide, being drawn through the apparatus during this time. Iodine

remains in the evolution flask as iodic acid; bromine passes off as such, and may be absorbed by allowing the air passing through the apparatus to bubble through 1% sodium hydroxide. Chlorine passes out of the evolution flask as chromyl chloride, and may also be absorbed in sodium hydroxide. Cool the mixture containing the iodic acid, and reduce the chromic acid by the addition of sulfur dioxide, about 20 cc. of a saturated solution being required. When a sufficient amount has been added the precipitated iodine redissolves, and the clear green color of chrome alum appears. Filter, wash the paper with water, dilute the filtrate and washings to about 300 cc., and add an excess of silver nitrate. Boil till the silver iodide has flocculated, allow to stand for a few hours, and separate and weigh the silver iodide in a tared Gooch crucible.

It sometimes happens that the mixture becomes turbid after the reduction with sulfur dioxide owing, apparently, to the separation of a basic chromium sulfate. Very often the turbidity can not be removed by filtering and it has been found advisable in this case to reject the determination and begin anew.

### SODIUM IODIDE.

(a) (Applicable with Erythrosine.)

Dissolve 5 grams of the dye in 400 cc. of water and add a mixture of 2 cc. of concentrated nitric acid (sp. gr. 1.42) with 10–20 cc. of water. Dilute to exactly 500 cc., mix and filter on a dry paper. Place 200 cc. of the filtrate in a porcelain casserole and make slightly alkaline with pure sodium hydroxide solution. Treat with an excess of 7% potassium permanganate solution and conduct subsequent operations as directed for the alkaline dye solution under Total Iodine, (a). However, when the total quantity of inorganic halogen present is small it is well to use correspondingly smaller amounts of the potassium permanganate and sulfur dioxide solutions.

### ETHER EXTRACTIVES.

(a) (Applicable with Naphthol Yellow S, Ponceau 3R, Orange I, Tartrazine, Amaranth, Light Green S. F. Yellowish and Indigo Disulfoacid.)

Dissolve 10 grams of color in 150 cc. of water and extract in a separatory funnel with ether that has been washed with water (using three 150 cc. portions of water for each liter of ether). Extract the color solution with two 100 cc. portions of this ether, shaking thoroughly for 1 minute, and wash the combined ether extract successively with 35, 20 and 10 cc. of water made alkaline or acid, as the case requires, with 1 cc. of 0.1 N alkali or acid per 100 cc. of water. Decant the ether from the mouth of the separatory funnel and rinse the latter once with 5 cc. of ether. The color solution is first extracted neutral, the extracted solution being then rendered alkaline with 2 cc. of a 10% solution of sodium hydroxide and again extracted with two 100 cc. portions of ether. In acidifying for the third extraction, add twice the amount of hydrochloric acid (1 to 3) necessary to neutralize the alkali, and repeat the extraction with two 100 cc. portions of ether. Place the neutral, alkaline, and acid extracts in a dust-free atmosphere and allow the ether to evaporate spontaneously, after which dry the residues to constant weight over sulfuric acid, using flat-bottomed dishes 2¾ inches in diameter, 1¼ inches in height, and of about 100 cc. capacity. The dishes should be thoroughly cleaned, wiped dry, and allowed to stand in a sulfuric acid desiccator for at least 2 hours before weighing. In order to avoid the generation of static charges of electricity, they should not be wiped immediately before weighing. Run two blank determinations with each series of ether extracts and deduct the average gain in weight of these two blanks from the weights obtained in the other determinations.

(b) (Applicable with Erythrosine.)

Determine as given under (a) omitting, however, the acid extraction.

DYE BY TITRATION WITH TITANIUM TRICHLORIDE.

(a) (Applicable with Amaranth, Ponceau 3R, Orange I, and Tartrazine.)

Prepare a standard 0.1 N ferric iron solution from pure crystallized ferrous ammonium sulfate, $(NH_4)_2Fe(SO_4)_2.6H_2O$. Weigh accurately 39.22 grams of the salt, transfer to a 1-liter graduated flask with 200–300 cc. of water and 30 cc. of pure concentrated sulfuric acid and agitate until the ferrous compound is dissolved. Weigh exactly 3.16 grams of pure crystallized potassium permanganate, dissolve in 100–200 cc. of warm water and add the solution slowly with stirring to the liquid in the flask. The permanganate solution should be exactly sufficient to oxidize the iron but it is well to add the last few cc. drop by drop. The iron solution, after having been treated with sufficient permanganate to show a faint but perceptible reddish tint, is cooled and diluted to 1 liter.

The standard titanium trichloride solution should be preserved in an atmosphere of hydrogen in an apparatus similar to that illustrated in Fig. 1. The reservoir bottle

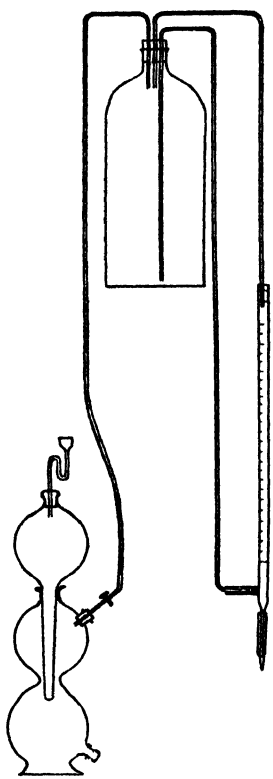

FIG. 1. APPARATUS FOR TITRATION WITH STANDARD TITANIUM TRICHLORIDE SOLUTION.

is connected by means of a syphon to the side neck of the buret. The buret is provided at the side neck tube and bottom with valves made from rather heavy red rubber tubing (of the very best quality) and carefully fitted glass beads. Glass tubes also connect the bottle with the top of the buret and with the Kipp hydrogen generator and reach about ½ inch below the rubber stopper of the reservoir. The tube leading to the top of the buret is connected with the latter by means of a tight rubber stopper.

The gas outlet tube of the Kipp apparatus is fitted with a well-greased glass stop-cock and connected by means of rather thick-walled rubber tubing to the glass tube leading to the bottle. The generator, which should be charged with stick zinc and dilute sulfuric acid, may be of 1-pint or 1-quart capacity. A 25 cc. buret and 2-liter reservoir bottle are of convenient capacity and the whole apparatus, including the generator, may be supported on a large, heavy ringstand by means of clamps and rings.

Prepare a dilute titanium trichloride solution by mixing 200 cc. of the commercial 15% solution with 100–200 cc. of concentrated hydrochloric acid and diluting with water to 2 liters. Measure exactly 10 cc. of the ferric ammonium sulfate solution into a small beaker and add a few grams of pure ammonium sulfocyanate or an equivalent amount of the concentrated solution. Fill a buret or graduated pipet with the titanium solution just prepared and add with stirring to the ferric sulfocyanate mixture until the color changes from red to pale yellow or colorless. Note the number of cc. of the trichloride used and add sufficient water or strong trichlorides as the case may require to bring the titer to between 0.100 N and 0.103 N. Transfer the solution to the reservoir bottle of the apparatus just described, which it must fill almost completely, insert the stopper and fittings into the bottle and wire down snugly, then fill the buret by applying suction at the top, the valve in the side tube being held open meanwhile. Care must be taken that all bubbles are carried out of the syphon tube. Insert the stopper at the top of the buret loosely and carefully open the stop-cock of the Kipp apparatus, allowing 100–200 cc. of hydrogen to pass over the solution and carry out most of the air. Insert the stopper in the buret and wire it down if necessary to make a tight joint. Finally open the stop-cock of the Kipp apparatus.

Before standardizing the solution it should be allowed to stand a day or so, so that any residual oxygen may be absorbed. The stock bottle should then be tilted back and forth a few times to eliminate any slight differences in concentration at the surface of the liquid and, after refilling the buret, the solution is ready for standardization.

Titanium trichloride solutions rapidly take up oxygen if not kept in well-stoppered bottles and commercial 15–20% preparations often contain rather large amounts of titanic compounds. When such partially oxidized solutions are used a white precipitate may form in the volumetric solution on standing. Although this does not affect the titer the precipitate may cause some inconvenience by clogging the valves. The formation of this precipitate may be avoided by using more hydrochloric acid in making up the solution but as this involves the use of larger amounts of sodium tartrate in some of the titrations a very strongly acid reagent is unsatisfactory.

Standardize the titanium trichloride solution by titration against the standard iron solution. Measure 20 cc. of the 0.1 N ferric ammonium sulfate into a 100 cc. flask, add 10 cc. of a 50% solution of pure ammonium sulfocyanate and titrate at room temperature in a slow current of carbon dioxide. The end point is shown by the disappearance of the red ferric sulfocyanate, the liquid becoming colorless or very pale yellow. The reduction is almost instantaneous but the last few drops of the solution should be added rather carefully. A rubber tube ending in a glass fitting which is hooked over the lip of the flask serves to conduct carbon dioxide from a bomb or Kipp generator over the surface of the liquid.

It is advisable also to determine the acidity of the titanium trichloride. Measure 5 or 10 cc. from the buret, add a measured excess of normal sodium hydroxide, allow to stand until the dark titanious hydroxide has oxidized to the white titanic compound, then add phenolphthalein or other indicator and titrate against standard acid.

Prepare a dye solution for the titration containing such an amount of the color that from 15–20 cc. of the standard trichloride will be required for its reduction. The quantities of each of the pure coloring matters reacting with 20 cc. of 0.1 N titanium trichloride may readily be ascertained from the table, page 216, and if these quantities of the crude dyes are taken for the titration the calculations are simplified. The volume

of the portion of dye solution taken for a titration should be 10–50 cc. Acidify the liquid by adding 1–2 cc. of concentrated hydrochloric acid, heat to boiling and boil for a few seconds in a current of carbon dioxide. Then remove from above the burner and, without interrupting the flow of carbon dioxide, titrate at once with the titanium solution.

Pyrex or similar Erlenmeyer flasks of 200 cc. capacity are convenient for this work as they may be held at the neck with forceps or a good spring hand clamp and heated directly over the free flame. The carbon dioxide is led from the bomb or generator through a rubber tube to a bent glass tube that hooks over the neck of the flask and conducts the gas below the surface of the dye solution. Burner, gas generator and titrating apparatus must be arranged within a few feet of each other.

No indicator is required in the titration as the end point is shown by the disappearence of the characteristic color of the dye, the mixture usually becoming colorless. The reduction takes place rather slowly in hydrochloric acid solution and, since the titanium compound readily absorbs oxygen, inaccurate results are usually obtained if it is added too slowly to the dye mixture. It is therefore advisable to determine the approximate amount required by a preliminary test. The reduction is more rapid with Amaranth than with Orange I, Ponceau 3R or Tartrazine, and the three last-named dyes can usually be estimated better in acid tartrate solution as described under (c). In making the dye titration the end point is easily overstepped so that it is of advantage to have a standard dye solution to titrate back any excess of titanium trichloride added accidentally or otherwise. Standard methylene blue solution is most convenient for this purpose. Amaranth and Light Green S. F. Yellowish are more readily available and it is recommended that standard solutions of these dyes (of 0.1 and 0.02 N respectively) be kept in stock bottles provided with capped, graduated pipets passing through the stoppers. The green will not be used for titrations in acid solution but is useful for certain determinations specified below under (c), (e) and (g). Because of the re-activity of the titanium trichloride, a large excess must not be added in making the determination in an open flask, as just described. If the amount of the standard dye solution required shows that an excess of trichloride greater than 0.3 cc. has been added the determination must be repeated.

(b) (Applicable with Naphthol Yellow S.)

Dissolve an amount of the dye corresponding to about 20 cc. of the titanium solution with a few cc. of water in a small flask provided with a rubber stopper and Bunsen valve. Add a slight excess of the titanium solution, prepared as described under (a). Pass a few hundred cc. of carbon dioxide over the surface of the liquid to displace air, stopper the flask and heat nearly to boiling for about 5 minutes. Then pass in carbon dioxide and titrate the excess of the reducing agent with standard methylene blue solution, or with standard Amaranth solution at boiling temperature, as indicated under (a). Naphthol Yellow S is more conveniently determined by titration in tartrate solutions, as given under (e) and (g).

(c) (Applicable with Light Green S. F. Yellowish and Indigo Disulfoacid.)

Proceed exactly as described under (a) except that the dye solution to be titrated is diluted to a volume of 60–75 cc. and treated with sodium acid tartrate instead of hydrochloric acid. Care must be taken on heating the solutions to boiling as they sometimes tend to foam at first. On this account the mixture should be cautiously boiled for a few moments before introducing the tube from the carbon dioxide generator. The amount of tartrate employed must be slightly greater than that required to react with the acid in the standard titanium solution used. The number of grams of crystallized sodium acid tartrate, $NaC_4H_5O_6 . H_2O$, equivalent to 20 cc. of the titanium trichloride is ascertained by multiplying the figure found for the acid normality of this solution by 3.8.

(d) (Applicable with Ponceau 3R, Orange I, Tartrazine, and Amaranth.)

Proceed exactly as described under (c). However, the solution should be standardized against pure samples of the dyes in question and the values so obtained used in the calculation instead of the values found with iron. The error introduced by using the iron value will tend to make the results too low but will not affect the figures more than 2% in the case of Ponceau 3R, Orange I and Tartrazine. With Amaranth the error is greater (perhaps 5 per cent) and as this presumably is due to side reactions, not well understood, it is advisable under ordinary circumstances to titrate this dye in acid solution by method (a).

(e) (Applicable with Naphthol Yellow S.)

Proceed as directed under (c) adding, however, 1 cc. of 0.2 N solution of Light Green S. F. Yellowish to the solution to be titrated to serve as indicator. Subtract 0.20 from the buret reading to correct for the effect due to the green.

(f) (Applicable with Yellow A. B. and Yellow O. B.)

Use a solution of from 0.1–0.2 gram of dye in about 50 cc. of 95% alcohol. Weigh off a slight excess of sodium acid tartrate, calculated as stated under (c), dissolve in about 50 cc. of boiling water, mix the solution with the alcoholic dye solution and titrate as directed under (d).

(g) (Applicable with Indigo Disulfoacid. Approximate values may also be obtained with Light Green S. F. Yellowish, Ponceau 3R, Orange I, Tartrazine, and Amaranth.)

Titrate exactly as stated under (c) and (d) using neutral sodium tartrate, however, instead of the acid salt. The amount in grams of crystallized sodium tartrate, $Na_2C_4H_4O_6 . 2H_2O$, equivalent to 20 cc. of the titanium solution is found by multiplying the acid normality by 2.3. The azo dyes show the irregularity noted under (d) to a slightly greater extent.

(h) (Applicable with Naphthol Yellow S.)

Proceed as directed under (g) adding, however, the indicator as used in (e).

(i) (Applicable with Erythrosine.)

Dissolve an amount of the product equal in weight to $\frac{1}{2000}$ mol. of the pure coloring matter (0.440 gram in case of Erythrosine) in 50 cc. of water, and add a quantity of neutral sodium tartrate equal to that which would be required to form free tartaric acid and sodium chloride with the hydrochloric acid in 20 cc. of the titanium trichloride. Warm until the salt has dissolved, then add 50 cc. of alcohol, heat cautiously to boiling in a stream of carbon dioxide and titrate in the usual way. As the reaction takes place rather slowly and the volume of the mixture is large in comparison with that of the trichloride required, the results ordinarily obtained are not very accurate.

*General statements concerning titrations with titanium trichloride.*—The procedures just described differ mainly through the variation in the acidity or hydrogen-ion concentration of the solutions in which the reduction is brought about. The reduction of the azo colors in hydrochloric acid solution appears to take place through a single reaction while at the lower acidity of the sodium tartrate and acid tartrate mixtures this is not strictly true. However, the reduction in hydrochloric acid takes place slowly while in the tartrate it is practically instantaneous so that in the latter case end points can be determined much more easily. In ordinary laboratory practice therefore the acid tartrate usually gives better results. The neutral tartrate gives in most cases less satisfactory results than the acid salt although it may be more suitable for dyes reduced with great difficulty. It is somewhat more convenient to use where applicable, as it is more soluble and but little more than half as much is required.

### DYE BY TITRATION WITH POTASSIUM PERMANGANATE.

(Applicable with Indigo Disulfoacid.)

Use a solution made by dissolving 0.200 gram of dye and diluting to 400 cc. Add 2 cc. of pure sulfuric acid and titrate against standard approximately 0.1 N potassium permanganate solution, the end point being shown by the production of a clear yellow color. As the amount of permanganate required for decolorization is some 5–10% less than that required by theory (assuming the formation of isatin-sulfonic acid) the titer of the standard solution must be fixed empirically by titration against indigo disulfoacid of known purity, the same conditions of concentration and acidity being observed.

### DYE BY COLORIMETRIC COMPARISON.

(a) (Methods applicable with all coloring matters that dissolve without decomposition and form stable solutions in aqueous or organic solvents.)

The graduated tubes or colorimeter employed for this examination must be so made as to enable the ratio between the thicknesses of the layers of the two solutions compared to be read within a few per cent of its value. It must also permit such adjustment as is necessary to eliminate any error due to unequal illumination or to color of the glass parts.

Prepare a solution of the coloring matter of such concentration that the predominant hue appears somewhat pale (due to admixture of white light) when viewed in a layer of thickness within the working limits of the comparison apparatus employed. Compare with a standard solution of known concentration in another portion of the same solvent. Concentration of the standard solution must be adjusted by preliminary trial if necessary so that it does not differ more than 20 per cent from that of the unknown. In reporting results obtained by this method, dye concentrations, solvent and type of apparatus should be stated.

(b) Proceed as described under (a) using, however, ordinary light modified by passage through colored glass or ray filters, in making the colorimetric comparisons. In general, the hue of the screen should be the complement of that of the solution.

(c) Proceed as directed under (a) except that the comparison is made in monochromatic light and with a colorimeter such as the Duboscq type permitting fairly accurate adjustment. The solutions must be of such concentration that they transmit only sufficient light to permit accurate visual comparison of the fields of the instrument and must in no case transmit more than 10% of the incident light. The light used must be of a wave of length for which the dye shows relatively high absorption. When results by this method are given the concentration of the solution, solvent, thickness of layer observed and wave length of light used should be indicated.

### DYE BY SPECTROPHOTOMETER.

(Methods applicable with all coloring matter that dissolve without decomposition and form stable solutions in aqueous or organic solvents.)

(a) (With monochromatic light source.) Procedure to be followed will depend upon kind of apparatus used. In reporting results give the extinction coefficient, with light of specified wave length, from which the dye concentrations were calculated.

(b) (With white light source.) Report results as under (a) including also the standard specific extinction coefficients used for comparison.

### LOWER SULFONATED DYES.

(a) (Applicable with Amaranth and Tartrazine.)

Dissolve a known amount of from 0.15–0.2 gram of the dye in 50 cc. of water and add 1 cc. of concentrated hydrochloric acid. Extract the solution by shaking out

successively in three separatory funnels each containing 50 cc. of amyl alcohol. Wash the amyl alcohol portions by shaking with 50 cc. of 0.25 N hydrochloric acid, which is passed in succession through the first, second and third funnels in the same way as the original solution. Repeat this washing operation with two further portions of 0.25 N acid. (One volume of concentrated hydrochloric acid (sp. gr. 1.20) diluted with 50 volumes of water gives an approximately 0.25 N acid.) Dilute the organic solvent with 1–2 volumes of gasoline and remove the water-soluble dyes by washing with several portions of water.

Estimate the separated dye by titration against standard titanium trichloride solution in the presence of sodium acid tartrate; or by a colorimetric procedure. State the procedure used for the estimation in reporting results.

(b) (Applicable with Ponceau 3R.)

Proceed exactly as described under (a) substituting, however, 50 cc. portions of amyl alcohol-gasoline mixture for the portions of amyl alcohol. The solvent is made by mixing equal volumes of amyl alcohol and low boiling point gasoline (sp. gr. 0.65).

(c) (Applicable with Indigo Disulfoacid.)

Carry out operations as described under (a) except that the original dye solution is treated with ¼–½ cc. of concentrated hydrochloric acid instead of 1 cc. and the washing acid is made 0.0625 N instead of 0.25 N.

(d) (Applicable with Light Green S. F. Yellowish.)

Prepare an acetate-salt mixture by treating a concentrated solution of sodium chloride containing 125 grams of the salt with a concentrated solution of 13.6 grams of crystallized sodium acetate $NaC_2H_3O_2.3H_2O$, adding 12 cc. of glacial acetic acid and diluting to 500 cc.

Dissolve 0.100 gram of the dye in 5–10 cc. of water, add 40 cc. of the salt-acetate mixture and extract the solution by shaking out successively in three separatory funnels each containing 50 cc. of amyl alcohol. Wash the amyl alcohol portions with three 50 cc. portions of the acetate-salt mixture, shake out each portion of washing liquid successively in the three funnels, and pass through the series in the same order as was the original solution. Remove the dye from the solvent and estimate as given under (a).

### MELTING POINT.

(a) (Applicable with Yellow A. B. and Yellow O. B.)

Determine by the procedure described by Gattermann.

## SPECIAL METHODS.

### MARTIUS YELLOW IN NAPHTHOL YELLOW S.

Dissolve 5 grams of the dye in 150 cc. of water, add 5 cc. of concentrated hydrochloric acid and shake vigorously in a separatory funnel for 1 minute with 50 cc. of petroleum ether or low boiling point gasoline. Separate the solutions and extract the aqueous liquid again with 25–30 cc. of the solvent. Combine the portions of gasoline, decant into a clean separatory funnel and wash with four 25 cc. portions of 0.25 N hydrochloric acid. Then remove the Martius Yellow by shaking with a few portions of dilute sodium hydroxide solution. Neutralize the alkaline dye solution with tartaric acid, add sodium tartrate if necessary and titrate against standard titanium trichloride as described for Naphthol Yellow S. Very small amounts may also be estimated colorimetrically (in neutral or slightly alkaline solution) by matching against a standard Naphthol Yellow S Solution, the tinctural power of the latter dye being considered as eight-tenths that of Martius Yellow. (The extraction procedure described is applicable with amounts of Martius Yellow below about 0.1 per cent.)

### BOILING POINT OF CUMIDINE FROM PONCEAU 3R.

(a) Dissolve 60 grams of the dye in a 600–700 cc. beaker with about 450 cc. of boiling water, add the hot solution very slowly to a warm solution of 100 grams of stannous chloride in 100 cc. of concentrated hydrochloric acid. The dye solution must be added 10–20 cc. at a time, waiting after each addition until the mixture has assumed a pale brownish color. If this is not done the dye will be precipitated and can then be reduced only with difficulty. The stannous solution should be at a temperature of 60–80°C. at the beginning of the operation. As reduction proceeds and the solution becomes more dilute the temperature is raised to boiling; care must be taken, however, that the mixture does not boil over after an addition of the dye as some heat is generated by the reaction. The reduction is carried out conveniently in a tall beaker of 1000–1200 cc. capacity. After all dye has been added and reduced, allow the mixture to cool and make alkaline by the addition of about 75 grams of sodium hydroxide dissolved in 150–200 cc. of water.

Cool the alkaline mixture and extract the cumidine by shaking it with two 200 cc. portions of ether. Combine the ether extracts and wash with water until the alkali and salts are removed. Evaporate the solvent on the steam bath but avoid prolonged heating that would volatilize appreciable quantities of the base. Fractionate the residue from a small side-necked flask, carefully avoiding overheating and collect separately the fractions; (a) boiling below 220°C.; (b) boiling between 220 and 225°C.; (c) boiling between 225 and 230°C.; and (d), boiling between 230 and 240°C. Weigh the fractions to within 0.05 gram.

### ORANGE II IN ORANGE I.

(Applicable when the proportion of Orange II is below 5 per cent.)

Dissolve 0.20 gram of dye in about 20 cc. of water and add 3–4 cc. of concentrated hydrochloric acid. Shake in a separatory funnel with 50 cc. of amyl alcohol, then draw off and discard the lower layer of liquid. Wash the amyl alcohol with six 50 cc. portions of N sodium carbonate solution made with such accuracy that it contains between 51 and 55 grams of the salt per liter. A second funnel containing 50 cc. of amyl alcohol is provided and each alkaline wash portion is drawn into this from the first separator and again extracted before being discarded. Finally wash the solvent in the second funnel with two additional 50 cc. portions of the carbonate solution.

Dilute the amyl alcohol portions with 1–2 volumes of gasoline and extract the dye by washing with several portions of water. Determine the dye by titration against standard titanium trichloride solution (in acid tartrated mixture) or by an accurate colorimetric procedure.

### IODEOSINE G IN ERYTHROSINE.

Mix 20 cc. of 0.020% solution of the dye with 20 cc. of concentrated hydrochloric acid, cool and shake with 25 cc. of ether. Compare with blank tests carried out in the same way with pure Erythrosine and with a mixture of 19 parts of this dye with one of Iodeosine G. With the pure Erythrosine the hydrochloric acid solution after extraction will be colorless and on addition of excess of ammonium hydroxide will remain so or show but a faint pink coloration. The acid used in the test with the mixture will be tinged yellow and will become red on the addition of ammonium hydroxide.

### ISOMERIC AND SIMILAR DYES IN AMARANTH.

Dissolve 0.100 gram of the dye in 40 cc. of water, add 10 cc. of 0.1 N benzidine solution (9.2 grams base per liter in 0.5 N hydrochloric acid), mix the solution well and allow to stand exactly 2 minutes. Filter through a folded filter and dilute 10 cc. of the

filtrate to 100 cc. Compare this solution colorimetrically with a standard Amaranth solution containing 0.40 mg. of the dye per 100 cc. If it is not more intensely colored than the standard solution the proportion of isomeric dyes may be considered to be below 1.5 per cent.

### SODIUM CHLORIDE IN DYES OR MIXTURES CONTAINING MORE THAN 25 PER CENT OF THIS SUBSTANCE.

An amount of the material should be taken for the determination that will contain 0.10–0.15 gram of sodium chloride. Treat the filtered solution with a slight excess of silver nitrate; add 5–10 cc. of concentrated nitric acid and an excess of 7% potassium permanganate solution (about 15 cc. per decigram of organic matter present). Boil the mixture for a few minutes, stirring to prevent bumping; then add sulfur dioxide solution slowly with stirring until the oxide of manganese dissolves leaving white silver chloride. Remove any excess of sulfur dioxide by boiling, cool and filter the silver chloride on a weighed Gooch crucible, wash, dry and weigh in the usual manner.

### SUCROSE IN DYE MIXTURES.

The procedure to be used will vary according to the nature of the other components of the mixture. Sucrose is readily soluble in saturated solutions of sodium chloride, sodium sulfate and potassium chloride and can be extracted from most dry dye mixtures with such solvents and estimated polarimetrically. The values found must be corrected by comparison with the readings obtained with solutions of known sugar concentration in the same solvent.

The barium salts of the color acids of indigo carmine, Naphthol Yellow S and Erythrosine are soluble with difficulty and that of Ponceau 3R is quite insoluble. Erythrosine is almost completely precipitated by lead acetate; Light Green S. F. Yellowish forms compounds of low solubility with $\alpha$-naphthylamine and similar bases.

## ADDENDA.

### SODIUM CARBONATE IN ERYTHROSINE.

(a) (Applicable with Erythrosine.)

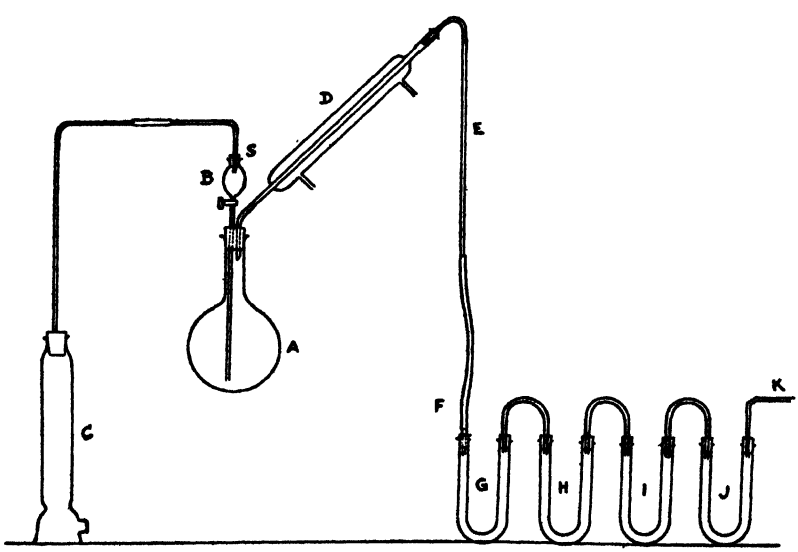


FIG. 2. APPARATUS FOR THE ESTIMATION OF CARBON DIOXIDE AND ERYTHROSINE.

Arrange the apparatus as illustrated in Fig. 2. The flask, *A*, is of about 600 cc. capacity and is fitted with a doubly perforated rubber stopper through which passes the stem of a small dropping funnel, *B*, reaching nearly to the bottom of the vessel, and the delivery tube of a short reflux condenser, *D*. The dropping funnel, *B*, is connected with a cylinder, *C*, by means of stoppers and glass and rubber tubing. The cylinder should be of about 200 cc. capacity and contain a 3-inch layer of soda lime held in place with glass wool or other suitable material. The condenser is connected by means of a rubber stopper and glass tube with a piece of rubber tubing, *F*, which can be connected either to the absorbing train or to an aspirator bottle or air pump. The absorbing train consists of U-tubes, *G*, *H*, *I*, *J*, connected by means of rubber stoppers and glass fittings. The U-tubes should be of such size that the arms are about 5 inches in length and ⅝ inch internal diameter. A rubber tube leading to the aspirator bottle or air pump is provided with two screw clamps or with some other device that will permit the rate of flow of the air drawn through to be readily and accurately adjusted, and is connected either to the last U-tube through the fitting, *K*, or to the rubber tube, *F*, through a short glass tube.

In order to make the determination, charge the absorbing train by measuring exactly 10 cc. of a standard approximately 0.1 N barium hydroxide solution into each of the U-tubes, adding a little boiled water when required so that in every case the lowest part of the bend of the U-tube will be completely filled with liquid.

Place 10 grams of the dye, a few glass beads and about 250 cc. of water in the flask, *A*, insert the stopper with its connections to the condenser and soda lime cylinder, close the stop-cock of the dropping funnel and heat the mixture to boiling. The absorption train is not connected with the apparatus during this operation. After 1–2 minutes, turn down the burner so that the solution boils but slowly, open the stop-cock of the separating funnel, attach the air pump connection to *E* and draw several liters of air through the apparatus to wash out the carbon dioxide. Then disconnect the pump from the tube, *F*, and insert the absorption train connecting it with the rubber tube, *F*, and with the pump through *K*. Draw 200–500 cc. of air through the apparatus, the dye solution being kept slowly boiling meanwhile. The barium hydroxide solution must remain clear, showing that all uncombined carbon dioxide has been removed. Disconnect the air pump at *K*, close the stop-cock of the separatory funnel, *B*, partially remove the stopper at *S* and pour a mixture of 4 cc. of concentrated sulfuric acid and 10–15 cc. of water into the bulb of the funnel. Replace the stopper, then open the stop-cock so that the acid may run into the dye solution. Then connect with the air pump again at *K* and draw air through the boiling liquid and absorption train, at first slowly, finally more rapidly until all carbon dioxide has been carried into the standard barium hydroxide. The solution in the last tube must remain clear.

Rinse the turbid portions of the standard solution into a 200 cc. Erlenmeyer flask with boiled water, close the vessel with a stopper and Bunsen valve or other device to prevent access of carbon dioxide, then heat the mixture to boiling to render the precipitated carbonate crystalline and less soluble. Finally cool and titrate carefully with standard hydrochloric acid using phenolphthalein as indicator.

## TABLE TO AID IN THE ESTIMATION OF DYES WITH STANDARD TITANIUM TRICHLORIDE SOLUTION.

Dyes are designated by the numbers given in "A Systematic Survey of the Organic Colouring Matters", 1904, by Arthur Green, based on the German of Schultz and Julius. Dye numbers enclosed in parentheses refer to closely related derivatives.

It is assumed that the titrations are carried out under such conditions that the dyes are reduced as stated by Knecht and Hibbert, "New Reduction Methods in Volumetric Analysis", 1918.

Molecular weights are calculated from the values adopted in 1920, oxygen being taken as 16.

TABLE 1.

*Estimation of the dyes with standard titanium trichloride solution.*

| DYE | MOLECULAR WEIGHT | AMOUNT OF 0.1 N TITANIUM TRICHLORIDE EQUAL TO 1 GRAM OF DYE | LOG (MANTISSA) | DYE EQUAL TO 1 CC. OF 0.1 N TITANIUM TRICHLORIDE | LOG (MANTISSA) |
|---|---|---|---|---|---|
| | | *cc.* | | *gram* | |
| *1* | 193.06 | 931.9 | 0.9694 | 0.001073 | 0.0306 |
| *3* | 256.11 | 468 1 | 0.6708 | 0.002134 | 0.3292 |
| *3* (+$H_2O$) | 274.12 | 437.8 | 0.6413 | 0.002284 | 0.3587 |
| *4* | 358.16 | 335.1 | 0.5251 | 0.002985 | 0.4749 |
| *7* (*Base*) | 197.17 | 202.9 | 0.3073 | 0.004929 | 0.6927 |
| *8* | 401.27 | 99.72 | 0.9988 | 0.01003 | 0.0012 |
| *9* | 429.32 | 92.76 | 0.9694 | 0.01073 | 0.0306 |
| *10* | 214.19 | 186.7 | 0.2712 | 0.005355 | 0.7285 |
| *11* | 248.19 | 161.2 | 0.2073 | 0.006205 | 0.7927 |
| *13* | 350.24 | 114.2 | 0.0577 | 0.008756 | 0.9423 |
| *14* | 452.29 | 88.41 | 0.9465 | 0.01131 | 0.0535 |
| *15* | 452.29 | 88.41 | 0.9465 | 0.01131 | 0.0535 |
| *16* | 225.21 | 177.6 | 0.2495 | 0.005630 | 0.7505 |
| *17* | 248.69 | 160.8 | 0.2064 | 0.006217 | 0.7936 |
| *18* | 262.71 | 152.3 | 0.1826 | 0.006568 | 0.8174 |
| *43* | 364.26 | 109.8 | 0.0407 | 0.009106 | 0.9593 |
| *44* | 466.31 | 85.74 | 0.9332 | 0.01166 | 0.0668 |
| *55* | 480.32 | 83.28 | 0.9205 | 0.01201 | 0.0795 |
| *56* | 494.34 | 80.91 | 0.9080 | 0.01236 | 0 0920 |
| *64* | 502.33 | 79.62 | 0.9010 | 0.01256 | 0.0990 |
| *65* | 502.33 | 79.62 | 0.9010 | 0.01256 | 0.0990 |
| *84* | 316.24 | 126.5 | 0.1021 | 0.007906 | 0.8979 |
| *85* | 350.24 | 114.2 | 0.0577 | 0.008756 | 0.9423 |
| *86* | 350.24 | 114.2 | 0.0577 | 0.008756 | 0.9423 |
| *87* | 327.26 | 122.2 | 0.0871 | 0.008181 | 0.9129 |
| *88* | 375.29 | 106.6 | 0.0277 | 0.009382 | 0.9723 |
| *89* | 477.34 | 83.83 | 0.9234 | 0.01193 | 0.0766 |
| *94* (*Na 2*) | 512.31 | 77.62 | 0.8900 | 0.01288 | 0.1100 |
| *94* (*Na 3*) | 534.30 | 74.85 | 0.8742 | 0.01336 | 0.1258 |
| *95* | 375.29 | 106.6 | 0.0277 | 0.009382 | 0.9723 |
| *101* | 400.28 | 100.0 | 0.0000 | 0.01000 | 0.0000 |
| *102* | 400.28 | 100.0 | 0.0000 | 0.01000 | 0.0000 |
| *103* | 502.33 | 79.62 | 0.9010 | 0.01256 | 0.0990 |
| *105* | 502.33 | 79.62 | 0.9010 | 0.01256 | 0.0990 |
| *106* | 604.38 | 66.18 | 0.8207 | 0.01511 | 0.1793 |
| *107* | 604.38 | 66.18 | 0.8207 | 0.01511 | 0.1793 |
| *108* | 706.43 | 56.62 | 0.7530 | 0.01766 | 0.2470 |
| *137* | 448.33 | 178.4 | 0.2515 | 0.005604 | 0.7485 |
| *138* | 556.37 | 143.7 | 0.1577 | 0.006955 | 0.8423 |
| *146* | 556.37 | 143.7 | 0.1577 | 0.006955 | 0.8423 |

TABLE 1.—Continued.

*Estimation of the dyes with standard titanium trichloride solution.*

| DYE | MOLECULAR WEIGHT | AMOUNT OF 0.1 N TITANIUM TRICHLORIDE EQUAL TO 1 GRAM OF DYE | LOG (MANTISSA) | DYE EQUAL TO 1 CC. OF 0.1 N TITANIUM TRICHLORIDE | LOG (MANTISSA) |
|---|---|---|---|---|---|
| | | *cc.* | | *gram* | |
| *150* | 380.31 | 210.4 | 0.3229 | 0.004754 | 0.6771 |
| *169* | 584.41 | 136.9 | 0.1364 | 0.007305 | 0.8636 |
| *197* | 419.23 | 190.8 | 0.2807 | 0.005240 | 0.7193 |
| *201* | 461.30 | 169.9 | 0.2302 | 0.005766 | 0.7609 |
| *240* | 696.51 | 114.9 | 0.0602 | 0.008706 | 0.9398 |
| *254* | 932.61 | 85.74 | 0.9332 | 0.01166 | 0.0668 |
| *269* | 554.33 | 144.3 | 0.1594 | 0.006929 | 0.8406 |
| *287* | 726.51 | 110.2 | 0.0419 | 0.009081 | 0.9581 |
| *319* | 992.65 | 80.60 | 0.9063 | 0.01241 | 0.0937 |
| *329* | 652.47 | 122.6 | 0.0885 | 0.008156 | 0.9115 |
| *372* | 780.53 | 230.6 | 0.3629 | 0.004336 | 0.6371 |
| *398* | 604.30 | 132.4 | 0.1219 | 0.007554 | 0.8781 |
| *427 (Chloride)* | 364.89 | 54.83 | 0.7390 | 0.01824 | 0.2610 |
| *427 (Oxalate)* | 926.94 | 43.16 | 0.6351 | 0.02317 | 0.3649 |
| *427 (Base)* | 346.44 | 57.75 | 0.7615 | 0.01732 | 0.2385 |
| *427 (hydr. Zn Salt)* | 1403.28 | 42.76 | 0.6310 | 0.02339 | 0.3690 |
| *433 (Na2)* | 730.60 | 27.37 | 0.4373 | 0.03653 | 0.5627 |
| *(433)* | 690.59 | 28.96 | 0.4618 | 0.03453 | 0.5382 |
| *435 (Na3)* | 832.65 | 24.02 | 0.3806 | 0.04163 | 0.6194 |
| *(435)* | 792.64 | 25.23 | 0.4020 | 0.03963 | 0.5980 |
| *447 (Chloride)* | 323.72 | 61.77 | 0.7908 | 0.01619 | 0.2092 |
| *447 ($Cl+4H_2O$* | 395.78 | 50.53 | 0.7035 | 0.01979 | 0.2965 |
| *451 (Penta)* | 393.83 | 50.78 | 0.7057 | 0.01969 | 0.2943 |
| *452* | 407.85 | 49.05 | 0.6906 | 0.02039 | 0.3094 |
| *452 (+8 $H_2O$)* | 551.97 | 36.23 | 0.5591 | 0.02780 | 0.4409 |
| *462 (Na Salt)* | 611.43 | 32.71 | 0.5147 | 0.03057 | 0.4853 |
| | 625.45 | 31.98 | 0.5048 | 0.03127 | 0.4952 |
| *464* | 567.51 | 35.24 | 0.5470 | 0.02838 | 0.4530 |
| *468* | 801.71 | 24.98 | 0.3975 | 0.04004 | 0.6025 |
| *479* | 797.59 | 25.07 | 0.3992 | 0.03988 | 0.6008 |
| *491* | 616.48 | 32.44 | 0.5111 | 0.03082 | 0.4889 |
| *504* | 478.85 | 41.77 | 0.6209 | 0.02394 | 0.3791 |
| *510 (Na Salt)* | 376.18 | 53.16 | 0.7256 | 0.01881 | 0.2744 |
| *510 (Acid)* | 332.20 | 60.20 | 0.7796 | 0.01661 | 0.2204 |
| *512* | 691.83 | 28.92 | 0.4611 | 0.03459 | 0.5389 |
| *516 (Na Salt)* | 628.00 | 31.85 | 0.5031 | 0.03140 | 0.4969 |
| *517 (Na Salt)* | 879.83 | 22.73 | 0.3566 | 0.04399 | 0.6434 |
| *517 (Acid)* | 835.84 | 23.92 | 0.3789 | 0.04179 | 0.6211 |
| *518 (Na Salt)* | 760.73 | 26.28 | 0.4197 | 0.03804 | 0.5803 |
| *520 (K Salt)* | 980.93 | 20.39 | 0.3094 | 0.04904 | 0.6906 |

TABLE 1.—Concluded.

*Estimation of the dyes with standard titanium trichloride solution.*

| DYE | MOLECULAR WEIGHT | AMOUNT OF 0.1 N TITANIUM TRICHLORIDE EQUAL TO 1 GRAM OF DYE | LOG (MANTISSA) | DYE EQUAL TO 1 CC. OF 0.1 N TITANIUM TRICHLORIDE | LOG (MANTISSA) |
|---|---|---|---|---|---|
| | | *cc.* | | *gram* | |
| *523 (K Salt)* | 1049.84 | 19.05 | 0.2799 | 0.05249 | 0.7201 |
| *523 (Na Salt)* | 1017.64 | 19.65 | 0.2934 | 0.05088 | 0.7066 |
| *584* | 350.74 | 57.02 | 0.7560 | 0.01754 | 0.2440 |
| | 364.76 | 54.83 | 0.7390 | 0.01824 | 0.2610 |
| *604* | 601.42 | 33.26 | 0.5219 | 0.03007 | 0.4781 |
| *639 (Chloride)* | 310.69 | 64.41 | 0.8089 | 0.01553 | 0.1911 |
| *650 (Chloride)* | 319.77 | 62.55 | 0.7962 | 0.01599 | 0.2038 |
| *650 (Zn Salt)* | 793.84 | 50.37 | 0.7022 | 0.01985 | 0.2978 |
| *692* | 466.28 | 42.87 | 0.6322 | 0.02332 | 0.3678 |
| *693* | 670.37 | 29.84 | 0.4747 | 0.03352 | 0.5253 |
| *Benzene-azo-b-Naphthylamine* | 247.21 | 161.8 | 0.2090 | 0.006180 | 0.7910 |
| *Toluene-azo-b-Naphthylamine* | 261.24 | 153.1 | 0.1850 | 0.006531 | 0.8150 |
| *Sodium trimethyl benzene-azo-b-naphthol-sulfonic acid.* | 392.29 | 102.0 | 0.0085 | 0.009807 | 0.9915 |
| *Sodium indigo sulfonate* | 364.23 | 54.92 | 0.7397 | 0.01821 | 0.2603 |

TABLE 2.

*Sulfur and sodium in some coal tar dyes.*

| DYE | SULFUR | SODIUM | SODIUM SULFATE CORRESPONDING TO SODIUM CONTENT |
|---|---|---|---|
| | *per cent* | *per cent* | *per cent* |
| Naphthol Yellow S | 17.91 | 12.84 | 39.67 |
| Ponceau 3R | 12.97 | 9.31 | 28.74 |
| Orange I | 9.15 | 6.57 | 20.28 |
| Tartrazine (Trisod. Salt) | 18.01 | 12.91 | 39.88 |
| Tartrazine (Disod. Salt) | 12.51 | 8.98 | 27.72 |
| Amaranth | 15.92 | 11.41 | 35.26 |
| Light Green S. F. Yellowish (Trisod. Salt M W 832.6) | 11.55 | 8.38 | 25.60 |
| Light Green S. F. Yellowish (Disod. Salt M W 792.6) | 8.09 | 5.06 | 15.62 |
| Erythrosine | 0.0 | 5.23 | 16.14 |
| Indigo Disulfoacid | 13.75 | 8.59 | 26.54 |

# REPORT ON METALS IN FOODS.

By W. F. CLARKE (Bureau of Chemistry, Washington, D. C.), *Referee.*

## ARSENIC.

The referee was instructed to rewrite the Gutzeit method in form for adoption as official. It was not possible to secure a copy of the printed revised methods in time to go over the arsenic method, as finally revised for printing, with reference to making any changes that might seem necessary before recommending its adoption as final. At the meeting of the American Chemical Society in April, 1919, H. V. Farr presented a paper, as yet unpublished, on the Gutzeit method which contained some valuable modifications of the present methods. In his procedure Farr returns to the older technique of passing the arsine against the sensitized paper instead of along a strip. The special apparatus which he uses is probably more satisfactory for tests where the chief question is whether or not the amount of arsenic exceeds a definite limit. Since a large amount of the work of this association is of this nature rather than the actual measurement of quantities which vary considerably, it is probable that Farr's method and apparatus should be given consideration with reference to adoption as official by this association.

## TIN.

Collaborative work has been carried out using the Penniman method[1] with the following modifications:

(1) For making the standard solution of potassium iodate a supply of the salt was used which had been dried at 120° to 130°C. for 3 or more hours. The solution was standardized against standard thiosulfate solution after the addition of hydrochloric acid and an excess of potassium iodide[2].

(2) Since potassium iodate with potassium iodide in the presence of hydrochloric acid yields free iodine, and also because iodine sufficient to color the starch was liberated in all the cases studied, potassium iodide was not added.

(3) The starch paste was made by adding 5 grams of thick cold water starch paste to 1 liter of boiling water and boiling for 2 additional minutes.

(4) Since all results obtained by most of the collaborators were very low, no blanks were run on the zinc. Blanks could only cause a reduction in the figures and the value of the blanks would be negligible.

(5) Since the expression "Titrate to strong blue color" is indefinite, the usual faint, but permanent, blue was used.

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[1] *J. Assoc. Official Agr. Chemists*, 1920, 4: 172.

[2] F. P. Treadwell and W. T. Hall. Analytical Chemistry. 5th ed., 1919, 2: 670.

## RESULTS.

Samples of standard tin solution and of composite, well-mixed canned pumpkin were furnished to all of the collaborators. The results obtained are shown in the following tables:

TABLE 1*.

*Collaborative results on standard tin solution.*

(Analyst, M. B. Porch, H. J. Heinz Co., Pittsburgh, Pa.)

| PENNIMAN METHOD† | | PENNIMAN METHOD‡ | | PENNIMAN METHOD§ | | BAKER-SELLARS METHOD** | | GRAVIMETRIC METHOD†† | |
|---|---|---|---|---|---|---|---|---|---|
| Tin present | Tin found | Tin present | Tin found | Tin present | Tin found | Tin present | Tin found | Tin present | Tin found |
| *mg.* | *mg.* | *mg.* | *mg.* | *mg.* | *mg.* | *mg.* | *mg.* | *mg.* | *mg.* |
| 41.67 | 41.90 | 41.67 | 44.60 | 41.67 | 39.40 | 41.67 | 41.00 | 41.67 | 43.90 |
| 41.67 | 42.50 | 41.67 | 44.60 | 41.67 | 39.70 | 41.67 | 43.00 | 41.67 | 45.20 |
| 41.67 | 43.70 | 41.67 | 44.20 | ..... | ..... | 41.67 | 44.00 | ..... | ..... |
| 41.67 | 42.10 | 41.67 | 44.90 | ..... | ..... | 41.67 | 43.75 | ..... | ..... |
| 41.67 | 43.60 | 41.67 | 44.90 | ..... | ..... | 41.67 | 39.55 | ..... | ..... |
| ..... | ..... | 83.33 | 86.50 | ..... | ..... | ..... | ..... | ..... | ..... |
| ..... | ..... | 83.33 | 85.30 | ..... | ..... | ..... | ..... | ..... | ..... |
| ..... | ..... | 83.33 | 89.11 | ..... | ..... | ..... | ..... | ..... | ..... |
| ..... | ..... | 83.33 | 86.90 | ..... | ..... | ..... | ..... | ..... | ..... |
| ..... | ..... | 83.33 | 87.50 | ..... | ..... | ..... | ..... | ..... | ..... |

*See remarks following Table 3.

†Starch and chloroform both used; end point read when starch showed strong blue and chloroform first showed pink.

‡Starch only used; end point read when starch showed strong blue.

§Starch only used; end point read when starch showed faint but permanent blue.

***Assoc. Official Agr Chemists, Methods.* 2nd ed., 1920, 150.

††*Ibid.*, 149.

TABLE 2.

*Collaborative results on standard tin solution*.*

| ANALYST | PENNIMAN METHOD | | | BAKER-SELLARS METHOD | |
|---|---|---|---|---|---|
| | Tin present | Tin found (starch indicator) | Tin found (chloroform indicator) | Tin present | Tin found |
| | *mg.* | *mg.* | *mg.* | *mg.* | *mg.* |
| A. E. Stevenson, National Canners Association, Washington, D. C. | 41.67 | 31.80 | ..... | 41.67 | 37.80 |
| | 41.67 | 31.30 | ..... | 41.67 | 37.20 |
| | 41.67 | 34.80 | ..... | 41.67 | 38.50 |
| | 83.33 | 65.10 | ..... | 83.33 | 74.30 |
| | 83.33 | 69.90 | ..... | 83.33 | 74.20 |
| | 83.33 | 75.50 | ..... | 83.33 | 75.00 |
| G. C. Spencer, Bureau of Chemistry, Washington, D. C. | 83.33 | 79.37 | 90.07 | ..... | ..... |
| | 83.33 | 78.02 | 90.37 | ..... | ..... |
| | 83.33 | 71.70 | 82.90 | ..... | ..... |
| | 83.33 | 75.24 | 85.50 | ..... | ..... |
| J. K. Morton, Bureau of Chemistry, Washington, D. C. | 83.33 | 76.30 | ..... | ..... | ..... |
| | 83.33 | 77.65 | ..... | ..... | ..... |
| | 83.33 | 80.70 | ..... | ..... | ..... |
| | 83.33 | 78.40 | ..... | ..... | ..... |
| | 83.33 | 78.60 | ..... | ..... | ..... |
| | 83.33 | 77.40 | ..... | ..... | ..... |
| R. M. Hann, Bureau of Chemistry, Washington, D. C. | 83.33 | 77.00 | ..... | ..... | ..... |
| | 83.33 | 76.80 | ..... | ..... | ..... |
| | 83.33 | 76.40 | ..... | ..... | ..... |
| | 83.33 | 76.80 | ..... | ..... | ..... |
| | 83.33 | 76.40 | ..... | ..... | ..... |
| W. F. Clarke | 41.67 | 38.20 | ..... | 16.67 | 16.87† |
| | 41.67 | 37.65 | ..... | 16.67 | 16.78† |
| | 41.67 | 38.00 | ..... | ..... | ..... |
| | 41.67 | 36.65 | ..... | ..... | ..... |
| | 41.67 | 37.25 | ..... | ..... | ..... |
| | 41.67 | 37.30 | ..... | ..... | ..... |
| | 41.67 | 37.90 | ..... | ..... | ..... |
| | 41.67 | 34.30 | ..... | ..... | ..... |
| | 83.33 | 71.10 | ..... | ..... | ..... |
| | 83.33 | 73.40 | ..... | ..... | ..... |

*Wherever starch was used as the indicator, the end point was read when the faint but permanent blue color was first obtained. Where chloroform was used the end point was read when the pink color disappeared.

†Results obtained after precipitation of the tin by hydrogen sulfide for 1 hour at room temperature, the sulfide being filtered on thick asbestos with gentle suction. When reducing the tin solution 150–175 cc. of concentrated hydrochloric acid (37%) were used.

## APPLICATION OF THE PENNIMAN METHOD TO THE ANALYSIS OF STANDARD TIN CHLORIDE SOLUTION.

Unless otherwise noted, in all the analyses by the Penniman method recorded in Tables 1 and 2, the solution was brought to a concentration of ammonium chloride and of free hydrochloric acid and to a volume

equal to that indicated in cases in which the hydrochloric acid extraction is necessary. After the adjustment of the concentration of the acid and of the ammonium salt, the method was followed closely unless exceptions to the contrary are noted.

TABLE 3.

*Collaborative results on the analysis of pumpkin.*

| ANALYST | PENNIMAN METHOD | BAKER-SELLARS METHOD | GRAVIMETRIC METHOD |
|---|---|---|---|
| | Tin found | Tin found | Tin found |
| | *mg.* | *mg.* | *mg.* |
| M. B. Porch | 44.30* | 44.00 | 39.40 |
| | 44.70* | 43.75 | 39.70 |
| | 41.00* | 40.00 | ..... |
| A. E. Stevenson | 43.63 | 50.37 | ..... |
| | 41.63 | 50.25 | ..... |
| | 40.63 | 47.75 | ..... |
| | ..... | 46.75 | ..... |
| G. C. Spencer | 36.30 | 46.52 | ..... |
| | 27.03 | 46.42 | ..... |
| R. M. Hann | 37.40 | ..... | ..... |
| | 40.20 | ..... | ..... |
| | 36.80 | ..... | ..... |
| | 40.70 | ..... | ..... |
| | 42.60 | ..... | ..... |
| | 43.00 | ..... | ..... |
| W. F. Clarke | 42.60 | 44.62† | ..... |
| | 42.35 | 46.40† | ..... |
| | 44.20 | 47.40† | ..... |
| | 44.03 | 47.95† | ..... |
| | 41.90 | 47.63† | ..... |
| | 41.68 | ..... | ..... |
| | 39.20 | ..... | ..... |
| | 38.20 | ..... | ..... |
| | 39.00 | ..... | ..... |

*Strong blue starch end point.
†Method modified as described for Clarke's results in Table 2.

NOTE.—In Table 3 generally 50-gram samples were used; otherwise all results are figured on the basis of 50-gram samples. In no case was the chloroform end point used.

## REMARKS.

With regard to the Penniman method, Porch states that he standardized the iodate solution against metallic tin; that he used generally 25 cc. of 31 per cent hydrochloric acid when precipitating the tin by zinc; that he tried to bring his titration end points to the same strong

blue shade in all cases, except as noted in Table 1; that he used as a chloroform end point the first pink that appeared; that he added the specified amount of potassium iodide in all cases; and that he found chloroform unsatisfactory in the pumpkin work.

Regarding the Penniman method, Stevenson notes that the results are low and non-concordant. Lacking time, he was unable to determine the causes of the discrepancy but thought the tin was probably incompletely reduced.

### ACCURACY OF THE STARCH END POINT IN THE OXIDATION OF THE STANNOUS CHLORIDE.

To test the usefulness of the starch end point as a mark of the completion of the oxidization of the stannous chloride by the iodate solution in the Penniman method, the referee carried out some determinations in which metallic tin and zinc were placed in the Erlenmeyer flasks, carbon dioxide passed through, the metals dissolved in hydrochloric acid and, after cooling, the stannous chloride was titrated with iodate solution.

TABLE 4.

*Oxidation of stannous chloride by potassium iodate.*

| ZINC | TIN PRESENT | TIN FOUND |
|---|---|---|
| *grams* | *mg.* | *mg.* |
| 3 | 30.80 | 28.90 |
| 6 | 30.60 | 26.80 |
| 3 | 38.10 | 36.70 |

It is seen from Table 4 that the starch end point does not mark the completion of the oxidation of the stannous chloride by the iodate solution, liberation of free iodine taking place prior to the completion of the oxidation.

### GENERAL DISCUSSION.

It is evident that better results are obtainable by the Baker-Sellars method than by the Penniman method in its present form. The one collaborator, Porch, who tried to make use of a strong blue end point obtained figures which were rather high in the case of the standard tin solution, whereas all results were low when the faint blue end point was used. It is believed that the concentration of free hydrochloric acid plays an important role in the completeness of the precipitation of the tin by the zinc. Probably such a quantity of acid should be used as will permit an excess of zinc to remain undissolved, thus preventing any tendency of the tin to go back into solution. Attention is called to the fact that the concentrated hydrochloric acid furnished recently

by the dealers seems to be quite variable as to the percentage of acid actually present, the values ranging between 31 and 37 per cent. In cases in which it is essential to have a definite concentration or in which only an acid of high concentration will serve, this irregularity may result in drawing wrong conclusions.

Possibly empirical conditions might be established which would permit of the use of the starch end point as a mark of the completion of the oxidation of the stannous chloride. Possibly a satisfactory way of using the chloroform end point might be found. Finally, the use of standard iodine solution might serve to make the method accurate. So far as the referee is concerned, however, it is not apparent that the method is more rapid in respect to actual manipulation net time as compared with the Baker-Sellars method.

### RECOMMENDATIONS.

It is recommended—

(1) That the Gutzeit method and apparatus for the determination of arsenic, as described by H. V. Farr, be studied and tested by collaborative work in comparison with the present Gutzeit method.

(2) That the tentative methods for copper and zinc[1] be studied by collaborative work and that any other apparently desirable methods be studied similarly.

(3) That the tentative volumetric method for tin[2] be made the subject of further study, with a view to modification, and that collaborative work thereon be conducted.

(4) That the Penniman method for tin be studied further with a view to revision or radical modification.

(5) That metals for which no methods have been studied by this association be studied in the order of their toxicity, the likelihood of their occurrence in foods being given first consideration.

## REPORT ON PECTIN IN FRUITS AND FRUIT PRODUCTS.

By D. B. Bisbee (U. S. Food and Drug Inspection Station, Old Custom House, St. Louis, Mo.), *Referee.*

A number of samples were sent out for collaborative work, together with copies of methods to be employed. Only one collaborator submitted a report and her results and those of the referee indicate that the methods employed, while recognizing the addition of pectin to fruits which do not normally contain any material amount of pectin,

[1] *Assoc. Official Agr. Chemists, Methods*, 2nd ed. 1920, 151.
[2] *Ibid.*, 150.

do not differentiate between the addition of pectin and the addition of so-called apple base.

RECOMMENDATION.

It is recommended—

That the methods for the detection of added pectin in fruit products receive further study.

F. B. Power (Bureau of Chemistry, Washington, D. C.) presented a paper on "The Detection of Methyl Anthranilate in Fruit Juices"[1].

## REPORT ON CANNED FOODS.

By W. D. Bigelow (National Canners Association, 1739 H Street, N. W., Washington, D. C.), *Referee.*

Your referee was asked to give special attention to methods for the detection of spoilage in canned foods. This subject has been studied but its complexity is so great that a report of progress is all that can be made at this time.

Apparently the question involves the bacteriological condition of the foods, chemical examination for the detection of the products of metabolism of spoilage organisms, and the critical examination of the containers. Much progress has been made in the development of methods along these lines for the determination of the cause of spoilage. Methods adapted to securing data of value to regulatory officers, however, involve some further complications. The following thoughts suggest themselves regarding such methods:

*Organoleptic methods.*—This type of method is one of the most important for the examination of canned foods. The analyst must be conversant with the food he is examining, must know its appearance, color, odor, and taste. An analyst thus qualified is better able to judge of the soundness of a sample by an organoleptic examination than by examination by means of bacteriological and chemical methods. The results of an organoleptic examination suggest questions which can often be answered by bacteriological or chemical examination.

*Bacteriological methods.*—When the organoleptic examination shows the product to be sound and when the can is found to be tight a bacteriological examination is usually superfluous, provided the product has been upon the open market and thus is known to have been canned long enough to permit bacteria which may be present to develop. When the organoleptic examination shows that the product is abnormal a bacteriological examination will often disclose the reason. When the

[1] *J. Am. Chem. Soc.*, 1921, 43: 377

product has an abnormal taste or odor, however, and is found to be sterile the microscopic examination of a hanging drop sometimes gives evidence of spoilage before sterilization. The presence of viable organisms in the sealed can does not prove that the product is undergoing spoilage. It not infrequently happens that bacterial spores are present whose resistance to heat is so great that they were not destroyed by the process to which the food was subjected but which do not germinate because of the acidity or of other characteristics of the product under examination. Again, the spores may only germinate at relatively high temperatures and thus, in ordinary storage, remain as spores indefinitely. The sterility of the sample, therefore, can not be made the sole criterion in judging of the soundness of canned food.

*Chemical methods.*—Certain products of bacterial spoilage, such as high acidity, may be detected by chemical methods. Since this question, however, is not peculiar to canned foods it would not appear to be appropriate to discuss it here.

*Examination of the can.*—Whenever a sample of canned food gives evidence of incipient spoilage by taste or odor, or when examined by bacteriological methods is found to be unsterile, a careful examination of the can should be made. By this it is not meant that the external appearance of the can should be noted. Leaks which cause spoilage in canned foods are not usually apparent from the outside. The seams should be filed and the construction of the can carefully noted. The procedure necessary in each case depends on the style of can and often on the character of the product canned. This procedure varies from time to time as changes are made in can manufacture and in methods of sealing cans. The technique required is difficult to explain and for that reason the analyst should receive personal instruction from one familiar with the industry.

No report on the physical methods of the examination of canned foods was made by the associate referee.

No report on tomato products was made by the associate referee.

## EFFECT OF THE USE OF DIFFERENT INSTRUMENTS IN MAKING A MICROSCOPIC EXAMINATION FOR MOLD IN TOMATO PRODUCTS.

By B. J. Howard (Bureau of Chemistry, Washington, D. C.).

The question has been raised from time to time as to what influence, if any, the form of sampling instrument with which the test drop is taken has on the percentage of fields containing mold in connection

with the microscopical examination of tomato products. The method[1] states that, "A drop of the product to be examined is placed on a microscopic slide and a cover glass is placed over it * * * ". Naturally, as a result of this vagueness, analysts used various instruments such as scalpels, penknives, glass rods, platinum loops, pipets, etc. In 1916, when the method was adopted as tentative the directions concerning this point were stated as follows[2]: "Remove the cover [of mold counting cell] and place, by means of a knife blade or scalpel, a small drop of the sample upon the central disk; * * * * ". Although this is more definite than the original statement, the question has been raised that the size and shape of the scalpel might have sufficient influence on the character of the drop to modify the count.

In order to study the question, two tomato samples were prepared as follows:

Unconcentrated cyclone juice was prepared by making a mixture of 95 parts of apparently sound tomatoes with 5 parts of rotten stock and pulping in a commercial cyclone. The second sample was made from a portion of the first by boiling it down to one-half of its original volume. The instruments used for taking the test drops were: (a) A large scalpel (blade about 40 mm. long); (b) a medium scalpel (blade about 31 mm. long); (c) a sharp-pointed scalpel; (d) a glass rod; (e) a platinum loop; (f) a pipet (touching the point to the slide); (g) a pipet (allowing the sample to drop freely from the point; and (h), the forefinger of the operator.

In order to eliminate so far as practicable the analyst variation the series of tests was made by each of three microscopists. In order further to eliminate the individual personal equation, three tests were made by each analyst by each method of sampling. In most of the cases each analyst made but one test by each method of sampling on any one day. Furthermore, none of the results were calculated till the data for the entire series had been obtained. Finally, the results were calculated and the average count by each method of sampling obtained.

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[1] U. S Bur. Chem. Circ. 68: (1911), 3.

[2] *Assoc. Official Agr. Chemists, Methods.* 1916, 324.

*Summary of*

| METHOD OF SAMPLING | UNCONCENTRATED JUICE | | | | |
|---|---|---|---|---|---|
| | Average of Analyst A | Average of Analyst B | Average of Analyst C | Group Results | |
| | | | | Average count | Variation |
| Sharp-pointed scalpel | 63 | 54 | 55 | 57 | 9 |
| Medium scalpel | 57 | 54 | 55 | 55 | 3 |
| Large scalpel | 51 | 55 | 56 | 53 | 5 |
| Glass rod | 43 | 54 | 61 | 53 | 18 |
| Pipet (touching slide) | 59 | 46 | 47 | 51 | 12 |
| Pipet (dropping free) | 56 | 53 | 59 | 56 | 6 |
| Platinum loop | 62 | 63 | 57 | 61 | 6 |
| Forefinger | 65 | 54 | 49 | 56 | 16 |

## DISCUSSION OF RESULTS AND COMMENTS.

From a study of the results, as shown by the final averages, it appears that the smallest average variations were obtained by the use of the medium and large scalpels. The average range between the results of the three analysts was 5.5 points. This was true of each sample of pulp considered individually. The widest range of results was noted where the glass rod was used for sampling, the variation being 17 points.

In using the pipet it was noted that more or less difficulty was experienced in getting the pulp to flow from the orifice which was only about ¾ mm. in diameter. This led to the conclusion that better results might have been obtained if a pipet with a larger orifice had been employed. In a separate set of tests it was further shown to be possible with a pipet to so manipulate the taking of the sample as to get very low counts on products which actually were high in mold, this variation being influenced by the manner in which the drop was released from the pipet. This fact argues strongly against this instrument for taking the drop.

In using the scalpels the sample was well stirred and the test drop taken by a quick, scooping motion during which the point of the instrument was plunged about 1 to 1½ cm. into the pulp and quickly raised, carrying with it a representative portion of the material which was quickly placed on the slide, previously cleaned and polished.

*mold tests.*

| CONCENTRATED JUICE | | | | | FINAL AVERAGES OF TWO GROUPS | |
|---|---|---|---|---|---|---|
| Average of Analyst A | Average of Analyst B | Average of Analyst C | Group Results | | Count | Variation |
| | | | Average count | Variation | | |
| 70 | 56 | 69 | 65 | 14 | 61.0 | 12.5 |
| 64 | 67 | 72 | 68 | 8 | 61.5 | 5.5 |
| 74 | 70 | 70 | 71 | 6 | 62.0 | 5.5 |
| 65 | 60 | 76 | 67 | 16 | 60.0 | 17.0 |
| 50 | 60 | 50 | 53 | 10 | 52.0 | 11.0 |
| 61 | 59 | 71 | 64 | 12 | 60.0 | 9.0 |
| 59 | 57 | 74 | 58 | 17 | 59.5 | 11.5 |
| 61 | 60 | 54 | 67 | 7 | 61.5 | 11.5 |

*President Lythgoe.*—It is the custom of this association to change the president every year, but we have one president who does not change. About twenty-three years ago I started doing food work, and either I had more time on my hands then than I have now, or my mind was more agile, but I read more of the work published by the agricultural chemists of this and other countries. I desired to get acquainted with the personality of the people who were doing this great work, and of all the men who were publishing work it seemed to me that the biggest one was our present Honorary President. It is possible that might have been due to youthful enthusiasm, but I am somewhat more mature now, and I am quite proud to state that I consider my youthful judgment to have been quite accurate, and I still consider our friend, the Honorary President of this association, the king-pin of the workers in this line. Friends, I take great pleasure in introducing to you our Honorary President, Dr. Harvey W. Wiley. (Great Applause.)

## ADDRESS BY THE HONORARY PRESIDENT.

H. W. Wiley (Good Housekeeping, Bureau of Foods, Sanitation and Health, Washington, D. C.).

Our friend, Mr. Einstein, discovered what he considered a new condition of affairs, but we all know that it was an old discovery for ever since we have been able to think we have understood that we measure all things by comparison with other things. Therefore, the doctrine of relativity is by no means a new one. I, especially, feel impressed with the truth of that doctrine as I look over this audience and compare it with the humble beginnings of this association. I believe there is one other gentleman present who, with me, was present at the original meeting of this association. It really began to bud in this city in 1880,

again the same year in Boston, and the next year in Cincinnati, but it was allowed to languish for some time until some of our friends in Georgia took it up and, in combination with the then Commissioner of Agriculture, J. T. Henderson, a meeting was called in Atlanta, Ga., in May, 1884. There, a more definite plan was talked over, and it was agreed to present this plan for final adoption at the meeting of the American Association for the Advancement of Science in Philadelphia in September of that year. We gathered around a small table. Electric lights were not known then; and stenographers were very scarce. We did not need any, by the way, to take down what was said because we took it down ourselves from mugs, various mugs upon the table. There is a good deal of relativity in that because mugs have now disappeared; we can only recall them by memory. E. H. Jenkins was the presiding officer at this meeting—known to fame as Jolly Jenkins. I wish he were able to come to this meeting. What a glorious thing it would be if Jenkins could walk in here and be greeted by you as you have greeted me! He was one of the great workers in the early days of this association, and W. J. Gascoyne, whom you all know, was there; and John Myers, who is dead and passed away; and Clifford Richardson, and C. W. Dabney, who afterwards grew to great proportions as a college president.

There is another way to compare this association, and that is by the men that it has produced, and the places where it has met. It is a long distance from the little hall in the Eutopian Club in Philadelphia to this grand place in the Willard Hotel. We are getting up in the world every year. I do not know where we are going to go after we leave here, but I suppose we will have our next meeting in the White House, and may be one of our men will be there because we have already sent one man to the Senate. So you see the doctrine of relativity is of some considerable importance.

I want to say a few words about some of the work that is now considered of prime importance along the line of your investigations—some work that I have not personally engaged in, except by following closely the workers and their results, but still work of wonderful magnitude. The work along the science of nutrition, which is peculiarly a work of this association, has gone forward by great strides in the last few years, and we are now beginning to see clearly the significance of it all. We have been seeking an *ignis fatuus* perhaps for several years in this line, trying to find some definite chemical formula for the vitamines, so-called. That quest is about ended. I think the consensus of opinion is now following the theory which I advocated some years ago that these bodies were mere accelerators or enzymes. The present opinion of investigators is that a vitamine is not a definite chemical compound, in the sense of the word that you can take out and measure

and determine what it is but that it is an infinitesimal element of matter, known as the accelerator or enzyme, which is really the vitamine that does the wonderful work in nutrition. If you take yeast, for instance (I am not an advance guard of Fleischmann or anything of that kind), which is very rich in a certain form of vitamine, and extract it, as I heard Atherton Seidell describe last Thursday night in his retiring address as President of the Washington Chemical Society, by adsorption you will find this product just as it was before; something has been removed of vital importance, of tremendous, vital importance, yet nothing ponderable has been removed. Hence, that is one of the reasons which led us to believe that it is an enzyme which does this work.

There is another relativity there. We have been sneering at our friends, the homeopathists for many years because they give nothing. They so dilute their remedies that there are practically none present and we say that you can not expect any great effect to come from nothing; nothing will produce nothing, according to the laws of nature. But after all, it may be that there is some virtue in the claims of our homeopathic friends, and that they may, if they can get hold of it, have a very dilute reagent which can produce wonderful results. When you consider that there is a difference between life and death in that one vitamine that prevents the development of beriberi, polyneuritis, and is the thing that is imponderable, so far as any means of our investigation are concerned, you begin to see the great value of the infinitely small. I once defined astronomy as the chemistry of the infinitely great, and chemistry as the astronomy of the infinitely small because one deals with infinitely large bodies and the other with infinitely small bodies, each of which is of equal importance in the cycle of life.

And so this vitamine which protects us against beriberi and polyneuritis, which is the same thing, is an imponderable substance. We can not expect to get it out of food and weigh it. We want to determine just what kind of an enzyme it is, and test the reactions of other digestive enzymes which attach themselves to these reagents. You can extract from the saliva the ptyalin; you can extract from the juices of the stomach the pepsin, and so forth. They all have the same chemical reaction as vitamines. This has been a wonderful investigation and of great importance to human nutrition.

And then the fact that the vitamine is of different kinds, which was not recognized by Funk and the early investigators, is of tremendous importance in human welfare. There are at least three kinds of vitamines; there may be more; we can not tell; we know that there are two or three or four different kinds of enzymes which proceed from the digestive functions. We know of the secretions from the endocrine glands which are of such vital importance to the growth and welfare of

the body. They are infinitely small; they are imponderable so far as our account goes. These are wonderful truths which have come to light by the investigations of men engaged particularly in your line of work, and this is only the beginning of this kind of investigation. In my opinion it will go still further and will accomplish still greater results for human welfare and human happiness than have already been obtained.

It is a remarkable fact that the lower animals have recognized the existence of enzymes and vitamines from time immemorial. The human animal is the only dumb animal in creation—almost—and it is the most helpless animal in creation. We pride ourselves about belonging to the *Homo sapiens*. I think we belong rather to the *Homo insapiens*. We are the last of the animals to appreciate the vitamines. When the bear or the wild animal kills another animal, he does not eat the first thing he sees. He seeks out the glands which have vitamines in them. He eats the heart and the liver and the pancreas and the kidneys first of all, and then if his hunger is satisfied he leaves the rest of the animal until the next day. He does not eat the flesh that we eat. We throw away the glands which have vitamines when we kill an animal. I always want the gizzard, at least, when you kill a chicken, but most people do not care for that. We eat the body first while the wild animal eats the glands where the fat-soluble enzymes reside. If there be any vitamines in the body at all they are in the fat that surrounds these vital organs of the body. That is the only part of the body that contains any vitamines of importance, and the animals always eat those parts first.

Seidell told of a curious experiment in feeding chickens with white flour and whole-wheat flour. They wanted to prepare it so that the chickens could eat it in the ordinary way, so they made a kind of spaghetti out of it, and they gave the chickens on the one hand these fragments made out of white flour, and those on the other side the fragments made out of whole-wheat flour. The chickens that ate the white flour all died of beriberi in thirty-five days, while the others were perfectly well at the end of that time. This is a great lesson in itself, but we do not heed it. We feed our children white flour, although we know it kills chickens, and we do not feed them whole-wheat flour, although we know it keeps the chickens alive. That is another evidence that man belongs to the *Homo insapiens* group. The question is, how much of this good food will protect the chickens that eat the bad food. That is an important physiological problem. So they began by mixing ten per cent of the good food with ninety per cent of the bad food. And what happened? These chickens, that have no intellect at all, picked out all of the fragments that came from the whole wheat side, and left all that came from the other kind. They did it instinctively in any mix-

ture that was given to them—they picked out the good food and left the bad food alone. So spaghetti was made out of the mixed flour, and in that way it was found that with about fifty per cent of the good flour the chickens could be protected pretty well against beriberi or polyneuritis. So you see these ideas of nutrition which we have just learned have been known by the lower, wiser animals from time immemorial. It is a great comment on human intelligence, is it not?

There is another line of investigation which lately came to my attention which I think is very interesting, and that is the investigation into botulism. I have just come from the Pacific Coast, and one particular thing which took me out there was to make a study of this problem at the source, and there I met these wonderful investigators who are going into this problem: one or two of them from the Bureau of Chemistry; the representatives of the two great universities in California,—the University of California and the Leland Stanford Jr. University; and the Bureau of Public Health which had one of its most important investigators out there joining in this work.

A great prejudice has been exercised or has arisen against eating ripe olives in the last year or two because of many deaths. I say many —I suppose ten or fifteen altogether—just what an ordinary automobile kills in Washington in a day, and nobody thinks anything about it, and nobody stops riding in automobiles, but when one person out of one hundred and five million dies from eating olives, everybody stops eating olives. That is another indication of how wise humanity is. We kill eleven thousand people in this country in a year with automobiles, and if ten people were to die of bubonic plague what a terrible scare there would be all over this country! These men have gone into this matter. First they looked over all the literature they could find, and found that this organism was called botulism because it was first found in sausage, and that was the Latin name for sausage. Cicero never used it; they did not have any sausage in those days so far as I know. We did not stop eating sausage, but we did stop eating olives. There are several remarkable things which are no longer secrets because they were told publicly at a dinner given to me there where all these men were invited to take part in the symposium. The best way to get at what was doing was to get each man to tell his story, and it was a most interesting story, and he told it straight from the shoulder. In the first place, this organism is probably as old, certainly as old as that which produces the lockjaw or tetanus, and exists like the tetanus germ in the soil; it is a soil organism. Of course, it is impossible to sterilize the soil, especially in a State that is a thousand miles long and three hundred miles wide; but they have found where the infected spots are and they are very particular that all foods grown in that region shall be subjected to the most careful scrutiny and sterilization in

order to make them perfectly safe for human consumption. They get into everything that grows in the soil. There is a great deal more botulism from green string beans than from olives, but green string beans, when they are opened, seem to reveal their character much better to the nostrils and, by the way, the odor of the toxin is the worst odor you can imagine. I never smelled anything that begins to compare in depravity with the odor of the poison produced by the Bacillus botulinus. It is something to remember for a long, long while after you get a whiff of it. But it does not appear in sound olives. They found that the most unsatisfactory environment for the growth of the organism is the olive in all stages, whether ripe or green, so long as it was not decayed; and it only grows in an olive when the spores are still stuck to the side of it, having come from the dust of the soil over which they grow. It only develops in an olive when it becomes decayed. Hence, if the canning is perfect, and the olive does not decay, the spore never grows. The toxin is the most violent poison known. They told me that if one cubic centimeter of the toxin were taken from the culture, and diluted one hundred thousand times, one cubic centimeter of that solution would kill a guinea pig, and hence one cubic centimeter of the poison would kill one hundred thousand guinea pigs. That might be of some service if it could be applied to rats and mice.

It is the most violent poison known but, at the same time, it is one of the most easily destroyed, because a temperature of boiling water will absolutely decompose this toxin and render it perfectly harmless. Hence, a simple precaution, if you have any doubt about the matter, would be to heat the suspected food to the temperature of 200 degrees or boiling water, but even then you would not want to taste it if you got a whiff of the material when heated. If such food is thrown out, it will kill chickens, or pigs, or anything that eats it. Sometimes the organism occurs in ensilage, in fodder and in hay that grows on this soil, and there has been a tremendous fatality among horses and cattle that eat spoiled ensilage and decayed fodder and hay from infected regions where botulism is known to exist.

It was a surprise to me that no green olives are packed in California. All of our green olives are imported; few imported ripe olives are used in this country. Our ripe olives are from California. All the crop not used for oil is put up as ripe olives in that State. The State Board of Health has gone into each ripe olive factory, and has installed an automatic register which runs for a week. It gives the actual temperature and the time in which the olives are kept in the sterilizer for every process that is carried on during that week. At the end of the week the agent of the Board of Health comes around and takes this record out; he has the only key. He then puts in a new sheet for the next week. In this way the State Board of Health has an automatic record

of the length of treatment or sterilization, and the temperature of the sterilization for every factory in the State. There are not many olive factories in the State, but those that do exist are very large. I spent a whole day in one of the largest factories in the State, going through step by step, every process in which the olives were treated.

So now, they claim the product of California to be absolutely safe. The thermal death point of the spore is about 240 degrees for forty minutes. No spore has been able to grow which has been subjected to that temperature for that length of time, and by this automatic register they can see what has been done to every batch of olives which is packed in California.

The scare because of the death of some ten or fifteen people in different parts of this country paralyzed the whole industry. The crop of the following year, that is, last year's crop, is left still in the hands of the packer. They are just beginning to move. The people are just getting over this scare, and the assurance that this crop has been packed with all these precautions will soon lift the ban and the business will regain its former vigor. If you are the least bit careful, if you open a can of ripe olives, and find a mushy one, throw the whole can away, because it may possibly be infected. There is not a very great probability, but there is a possibility. The firm olive, the perfect olive, is absolutely safe, and it is a splendid food and has a most delightful flavor for people who once learn to eat it. It is a great business and is now on a perfectly safe foundation.

All this has been done by chemical and bacteriological research. Every step has been conducted by the union of these two sciences, and the Bureau of Public Health, the Bureau of Chemistry and the University of California, and the Leland Stanford Jr. University. The National Canners Association advanced the money. It is a wonderful thing to see the business men of this country so deeply interested, not only in the financial part of their business alone, but in the excellence of their product. That is one of the most hopeful signs that has grown out of all the agitation which you and I have made in this country for better food products, and all of this, my friends, has grown out of your work and the work which has been done in the last twenty-five or thirty years to put the food products of this country on a higher plane and the people who are making these food products begin now to appreciate the work that has been done.

A short time ago I was standing at the Grand Central Station in New York City and a gentleman in front of me, after buying his ticket, said: "Are you Dr. Wiley?" I replied that I was. I did not know what he was going to do with me, but I was ready to take the consequences. He continued: "I am so glad to see you; give me your hand. A few years ago I thought you were the devil incarnate; I am the Presi-

dent of the Long Island Oyster Growers' Association, and when you told us that we could not take oysters out of certain infected localities, and could not ship them in the old way, soaking them in water and packing in ice, we thought you were our bitter enemy and that you were determined to destroy our business. If I had seen you then I would have expected you to have horns and a long tail, I was so impressed with the fact that you were the devil himself but you have done more for our industry than any other man in the world. We are selling five oysters now to one then, and we are sending oysters to all parts of the country and they are just as good when they get there as when they leave the water, all due to the fact that you dealt us what we thought was a death blow to our industry."

It has not been so many years ago that I was threatened to be mobbed by the forerunner of the National Canners Association; they were going to do me violence. I was invited to speak before that association at Atlantic City and when the president met me at the station, I said: "What is the matter? Are you ill?" He looked like he had been eating Atlantic fresh fish and ice cream. The fish came from Rhode Island, and the ice cream was made last year, and when you put the two together it is pretty bad; once I ate some of it and I felt just like he looked. "No," he replied, "I am scared." Whereupon I asked him what was the matter and was told that they were going to mob me. He advised me to take the next train and go home. I had gone up there to make a speech, and if I do not make a speech I am miserable, and if I do my audience is miserable, so there you are; fifty-fifty. I continued: "Are these men American citizens?" "Yes," was the reply. "Are they church members, and are they mostly republicans?" "Yes," he answered "They are mostly republicans." Then I said: "I do not believe I will be afraid to face an audience of Americans who are church members and republicans; I will take the risk. But is there a door at the back of the stage?" He told me that he thought there was. I asked if it were open and he promised to see that it was. He took me through the back door, up the back stairs, and into his room, saying: "You do not dare go into the lobby of this hotel; they will do you physical violence". There was a vast audience; men were standing, and did not look very favorable from their countenances. A great, big two-hundred pounder stood by the door and when I went in said: "We are not going to do a thing to you". I replied that I hoped not. The president was absolutely afraid of physical violence because I had told these men that they were putting out adulterated and misbranded foods, and they had to quit it. That was before the Food and Drugs Act was passed, and I had only moral force behind me. The president said: "You are the first after luncheon, but the man who follows you is going to read a long, prosy paper; let us put him in first". I said I realized the power

of personal pulchritude over an angry audience, and perhaps if I got up there and let them look at me for half an hour they would not be so cantankerous. So, they put him up and it was the shortest half-hour I ever experienced. My time came quickly. I got up amid a deathly silence. I speak a great deal and I am used to getting a little applause when I first get up; I hardly ever get any when I sit down, so I appreciate that which comes first. There was absolutely the silence of death in that vast audience, and I looked at them in my mild, benignant way. I have a ministerial look, and have been taken for a minister of the gospel. A few years ago, when I was young and handsomer and wore a Prince Albert coat instead of this next year's cut that I am wearing now, I went to visit the Girard College in Philadelphia, and the gatekeeper stopped me saying: "You can not enter here; in Stephen Girard's will it is provided that no minister of the gospel shall ever step inside of this enclosure". I looked at him in my mild, benignant way, and said: "The hell you say!" and was told to "Walk right in, sir; walk right in".

I looked at that audience in that same benignant spirit that I have just described and began very gently: "If there is a man in this audience who would put his hand in his neighbor's pocket and take out a dollar that does not belong to him and put it in his own pocket, stand up." Nobody stirred. And then I tried another tack on them. "Is there a man in this audience who would so degrade a case of canned goods that he puts up and so adulterate and misbrand them that he could sell them for a dollar more than they are worth, and put that dollar in his own pocket, will he stand up?" That is what every last mother's son of them had been doing, but not a single man stood up. And someone away back in the audience started a little applause, and it grew and became a roar of approval. They were honest men; they were American citizens; they were republicans as well as democrats, and then—I say then,—I did not say now, but then they knew that they were convicted and they recognized it. I went on and told them some of their shortcomings that still had to be righted, and when I got through sat down in a thundering applause, and the man who touched me on the shoulder and said: "We are not going to do a thing to you" arose. "Mr. President, I move a vote of thanks to Dr. Wiley for his most enthusiastic and encouraging address." It was carried unanimously. That became the National Canners Association that is doing this great work. That is where they were convicted and converted. Why, St. Paul's conversion is not much better than that, and I had nothing to do with converting St. Paul, I can assure you, but it was just as radical and overwhelming, and since that day that association has followed that lead, and today it is one of the most ethical associations, and doing that splendid work, raising money and employing these experts, many

of them former members of the Bureau of Chemistry. It is almost like going to the Bureau of Chemistry to go around and visit these establishments; boys, old boys, and new boys, boys that have come into the Bureau since I left it, and have gone out into this great industry, to do something for the food products of this country.

Gentlemen, I am glad to have had this opportunity to say a few words about the progress you have made and the relations which exist between this association today and at the time of its inception, and of the wonderful future which is before you in all the varied industries of this country. Not only food, but every other industry will seek and demand your services in order that the industries may be put upon the ethical basis of doing the best possible for those who consume the products of industry. Is not that a wonderful thing to think about? Is it not wonderful to show the progress which has been made? (Prolonged applause.)

*R. N. Brackett.*—I would like to move a rising vote of thanks to Dr. Wiley for his address.

*President Lythgoe.*—The chair is pleased to request a rising vote of thanks to Dr. Wiley. (Prolonged applause.)

*Dr. Wiley.*—Thank you, Mr. President.

*President Lythgoe.*—We can go a long way before we can find a scientific man who can get up and give us such an extemporaneous address.

## ADDRESS BY THE SECRETARY OF AGRICULTURE—THE HONORABLE E. T. MEREDITH.

I feel that Dr. Alsberg owes this group an apology for inflicting me upon you, but it is a pleasure to me and a privilege to run in for a moment and assure you of my personal appreciation and the appreciation of the Department of Agriculture of the value of the work you are doing. There is no question that today research work along all lines has come to be more fully appreciated and valued by the people generally. There are many things that have brought this about. The world war has added to our knowledge of what may be accomplished in the way of research. The advancement that has been made in the study of foods, the study of fertilizers, and many other lines has added to that. It was not so long ago, as I am sure you appreciate, that many of the research men and the scientists were referred to quite generally as "long-haired scientists". I have heard the reference many, many times when in contact with agricultural groups and others. I do not see any here who would qualify as long-haired scientists, but such comment is passing now, and I feel that I may say to you earnestly and sincerely that there is no group of workers in the country whose

work is more fully valued and appreciated than that of the research workers. It has been my privilege during the past few months to emphasize this work somewhat and in talking with groups of farmers I have emphasized research and the value of the work of the scientist. I have asked farmers if they believed in the rotation of crops, and they have said, of course they believed in the rotation of crops; and then I have asked them who told them what a crop, a particular crop might need in the way of plant food; who told them what elements of the soil went to make certain crops; who named the elements of the soil, and so on. And they came to see that the rotation of crops was really based upon the research work of the scientist, the analysis not only of the soil, but of the crop; and then I have asked them if they believed in balanced rations for their live stock, and in every case they said: "Why, certainly, we practice balanced feeding; that is practical agriculture". And then when you ask them what elements go to make milk, what elements go to make beef, and who named those elements in the feed, and who named the elements in the product, then they appreciate that it was a scientist again, a man who had analyzed, who had studied, and they come to believe and to appreciate that it is all based in the final analysis upon scientific study and research work.

So I feel that you may be perfectly satisfied and take great pride in the fact that the value of this work is becoming more and more generally appreciated and its relations to all our activities, not alone in agriculture, to which you men and women are devoting your particular studies, but to all lines of endeavor recognized.

We are finding in the Department of Agriculture that this is true in another way than simply expression of appreciation on the part of the people generally, but in the fact that large commercial organizations are constantly calling upon the Department for men, offering them more attractive salaries than we have been privileged so far to offer, and taking them away from the Department to devote their time and energies to particular institutions to the credit and the advantage of these institutions. So to you who are doing this great work, I at least want to say for myself, that I feel you are doing a public service, and that you are to be most heartily commended. I feel, too, that the material advantages should be largely increased to those who are doing this worth-while work in the study of foods, the study of all the things that go for our material comfort and our health. That means better salaries. This would mean more opportunity for the young men and would attract more young people to this line of work. I hope that through our efforts, particularly with Congress, there will be greater attractions in the Government service along this line in the future than there have been in the past.

You in your particular line of work have accomplished much in the methods of analysis and examination, but I am sure you understand, and that I may be privileged to say, that there is still much to be done, and that those young in the work and your members who are young in years, will have just as many opportunities for real accomplishment and will find just as many responsibilities as those who are older in the work and have carried the burdens up to this point. There is much in the study of foods, in our fabrics, in our fertilizers and other lines to be done, and certainly the members of this organization must play a great part in that work. I wish to congratulate you and commend you most heartily for what you have done, and wish for you still greater accomplishments in the future. I thank you.

The meeting adjourned at 12:45 p. m. to reconvene at 2 p. m.

## SECOND DAY.

## TUESDAY—AFTERNOON SESSION.

### REPORT ON CEREAL FOODS.

By C. H. Bailey (Agricultural Experiment Station, University Farm, St. Paul, Minn.), *Referee.*

The referee received his appointment, and necessary reports and other data too late to make possible the completion of the desired collaborative work. Moreover, at the time of the receipt of this material only one collaborator had signified a willingness to assist in this work, and considerable time was consumed in correspondence with prospective collaborators. A half dozen chemists have agreed to collaborate, outlines for work in accordance with the recommendations of Committee C[1] have been drawn up, and samples are being prepared and distributed to the collaborators.

The American Institute of Baking has, during the past few months, conducted a study of methods employed in the analysis of flour, to determine how nearly a number of chemists checked one another when analyzing the same material. Three samples, marked A, B, and C, were sent to each of the collaboratoring chemists in rubber-stoppered bottles, with the request that the percentage of moisture, ash, crude protein, and crude gluten be determined by the method usually employed in their respective laboratories. The American Institute of Baking has kindly furnished tabulated statements of the several reports, which are worthy of careful scrutiny.

In the case of moisture determinations, so far as the referee can discern from the reports, only 12 of the 28 chemists who reported are employing the official method, which provides for drying to constant weight in vacuo or hydrogen. The other 16 are apparently using air ovens heated in a variety of ways. In more than half of the cases where air ovens were used the temperature was maintained appreciably above 100°C., namely, from 103° to 105°C.

In Table 1 are given the maximum, minimum, and average moisture percentages as determined by the official vacuum oven method, and by drying in air ovens. The single report on drying in hydrogen is not included in this summary. These data show: 1st, somewhat higher averages where the vacuum oven was used; and 2nd, a tendency towards smaller deviations from the mean when the results by the use of the

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*J. Assoc. Official Agr. Chemists*, 1921, 4: 577.

vacuum oven are compared with those obtained by drying in contact with the air. This tendency is emphasized still further by omitting from the series of Samples C, which was dried in vacuo, the report of 10.73 per cent, which is distinctly out of line with the other reports on these samples. This omission would reduce the range from maximum to minimum in the samples of flour C dried in vacuo to 1.27 per cent instead of 2.61 per cent as shown in Table 1.

TABLE 1.

*Determination of moisture in flour samples.*

| METHOD | SAMPLE A | SAMPLE B | SAMPLE C |
|---|---|---|---|
| | *per cent* | *per cent* | *per cent* |
| Dried in vacuo | | | |
| Maximum | 12.83 | 11.16 | 13.34 |
| Minimum | 11.76 | 10.25 | 10.73 |
| Average | 12.37 | 10.88 | 12.81 |
| Dried in air ovens | | | |
| Maximum | 12.80 | 11.50 | 13.59 |
| Minimum | 10.70 | 9.03 | 11.08 |
| Average | 11.93 | 10.53 | 12.53 |

These results seem to emphasize the desirability of adhering to a standard procedure in the determination of moisture, and to the elimination of the air oven in favor of the vacuum oven.

Ash was determined in each of the three flour samples by the same 28 chemists. Table 2 gives a summary of their reports. While a fairly wide range is shown in the percentage of ash as determined by different chemists, by scanning the individual reports it is seen that in 26 out of 28 reports on Sample A there was a deviation of 0.02 per cent or less from the mean, and in 25 out of 28 reports on Samples B and C the deviation from the respective means was no greater. Three of the analysts reported the use of calcium acetate in the incineration of the flour. The averages of these three determinations were: Sample A, 0.445 per cent; Sample B, 0.380 per cent; and Sample C, 0.693 per cent. There appeared, accordingly, a slight tendency towards higher results by the use of calcium acetate in the determination of ash.

TABLE 2.

*Determination of ash in flour samples.*

| DETERMINATION | SAMPLE A | SAMPLE B | SAMPLE C |
|---|---|---|---|
| | *per cent* | *per cent* | *per cent* |
| Maximum | 0.470 | 0.390 | 0.730 |
| Minimum | 0.403 | 0.324 | 0.670 |
| Average | 0.427 | 0.350 | 0.696 |

Protein determinations were made in all cases by the Kjeldahl method or one of its modifications. The results show some wide variations due, apparently, to various causes. At a recent meeting of cereal chemists it seemed to be the consensus of opinion that where several analysts failed to obtain the same results in the determination of crude protein, errors were to be attributed generally to inaccurate standardization of the acid solutions used in titration. That this is not the sole cause of variations in the reports on these three samples is to be deduced from the fact that the analyst who reported the lowest percentage of crude protein in Sample A also reported the highest percentage in Sample B.

TABLE 3.

*Determination of crude protein in flour samples.*

| DETERMINATION | SAMPLE A | SAMPLE B | SAMPLE C |
|---|---|---|---|
| | *per cent* | *per cent* | *per cen* |
| Maximum | 13.10 | 10.00 | 12.84 |
| Minimum | 11.48 | 8.31 | 11.28 |
| Average | 12.14 | 9.21 | 11.97 |

To ascertain whether the standard acid used by the several analysts varied sufficiently to account for the differences in the results reported, the data were divided into two groups. The basis of division was the percentage of crude protein reported in Sample A. In all instances where the analyst reported less than the average percentage of protein, his data were averaged by samples and constituted the first group. Where the percentages of protein reported in Sample A were greater than the average, the data for all three samples were grouped by samples, and constituted the second group. In Table 4 the averages of the groups divided in this manner are shown. These averages indicate that the analysts who reported higher percentages in Sample A also reported, on the average, higher percentages in Samples B and C, although the results are not in direct ratio. Moreover, there are individual exceptions to the general rule. It may be concluded that while errors in standardizing the acid used in titration are probably responsible for certain of the deviations shown, other errors occur at the same time.

TABLE 4.

*Average group determination of protein.*

| GROUP DETERMINATION | SAMPLE A | SAMPLE B | SAMPLE C |
|---|---|---|---|
| | *per cent* | *per cent* | *per cent* |
| Where protein reported in Sample A exceeded the average | 11.85 | 9.03 | 11.86 |
| Where protein reported in Sample A was less than the average | 12.39 | 9.37 | 12.09 |

The results of the determination of crude gluten by 21 analysts varied more than did the determination of crude protein. This is doubtless to be expected, in view of the character of the method. In Table 5 are shown the maximum, minimum, and average percentage of crude gluten reported by 21 analysts. It is interesting to note that in the case of the two flours of fairly high gluten content the average percentage of crude gluten, and of crude protein (chemically determined) are almost identical. In the case of Sample B, which contained a low percentage of gluten, the average crude gluten was appreciably less than the average crude protein.

TABLE 5.

*Determination of gluten in flour samples.*

| DETERMINATION | SAMPLE A | SAMPLE B | SAMPLE C |
|---|---|---|---|
| | *per cent* | *per cent* | *per cent* |
| Maximum | 13.50 | 10.60 | 13.00 |
| Minimum | 9.80 | 5.90 | 10.00 |
| Average | 12.00 | 8.65 | 12.06 |

This work, which was conducted by the American Institute of Baking, is of significance in that it emphasizes the necessity of uniformity in procedure, and also indicates the relative deviation from the means which may be anticipated when determinations are made by a number of analysts.

The referee regrets that another organization has undertaken to promulgate standard methods for the analysis of cereal products. Where the official or tentative methods of this association are thus prescribed no particular harm is done, and neither does any advantage accrue. The promulgation of other than official methods of this association by an association of chemists seems regrettable, however, and something to be avoided.

RECOMMENDATION.

The referee recommends that work now in progress, recommended by Committee C in 1919[1], be continued.

No referee on the subject of distilled liquors was appointed and no report on this subject was presented.

No referee on the subject of wines was appointed and no report on this subject was presented.

No report on the limit of accuracy in the determination of alcohol in beers was made by the referee.

[1] *J. Assoc. Official Agr. Chemists*, 1921, 4: 577.

No referee on methods of analysis of near beers was appointed and no report on this subject was presented.

No general report on vinegars was made by the referee.

## A NOTE ON THE POLARIZATION OF VINEGARS.

By R. W. BALCOM and E. YANOVSKY[1] (Bureau of Chemistry, Washington, D. C.).

In dry cider and in cider vinegar there are usually at least three optically active substances present, namely, sugar (levulose), malic acid, and lactic acid[2]. If the fermentations have been normal the rotation of cider and of cider vinegar is never plus, so far as is known, although it may approach zero as a limiting value from the negative side. Abnormal or unusual fermentations sometimes, though rarely, occur. An instance of such fermentation in a cider was observed in 1910 by the Bureau of Chemistry when, in the course of its regulatory work, a sample of cider was collected which, upon analysis, was found to be dextrorotatory. Further investigation showed that it was a genuine product in which the levulose had been fermented out before the dextrose, instead of the dextrose before the levulose as is ordinarily the case. The usual procedure in determining the polarization has been to clarify with solutions of neutral lead acetate or of basic lead acetate and polarize without the removal of lead from the filtrate. The directions given in the methods of this association call for the use of basic lead acetate supplemented, when necessary, with alumina cream.

In 1912 Bender[3] reported having found a plus polarization of 0.7°V. for a sample of cider vinegar when this vinegar was clarified with lead subacetate in the usual way. The vinegar in question had been manufactured in his presence so that there was no question as to its authenticity. It contained an unusually large quantity of nonvolatile acid, 0.32 gram per 100 cc., calculated as malic acid. The same vinegar, when treated with bone black alone, gave a negative polarization of 0.44°V. Bender concluded that this anomaly might be due to the effect of lead salts in solution. His experiments with malic acid and lead subacetate confirmed this assumption and in his recommendations as referee he suggested a study of the method used for determining polarization.

In 1915 a sample of vinegar found on the market labeled as apple vinegar was examined by the Philadelphia Food and Drug Inspection Station of the Bureau of Chemistry. The analytical results obtained

[1] Present address, Norwalk Tire and Rubber Co., Norwalk, Conn.
[2] *J. Ind. Eng. Chem.*, 1917, 9: 759.
[3] U. S. Bur. Chem. Bull. 162: (1913), 81.

were normal for a cider vinegar except that like the Bender sample its nonvolatile acid content was high (0.31 gram per 100 cc.) and it gave a plus polarization of 0.56°V. This sample when further examined in the Food Investigation Laboratory of the Bureau of Chemistry, by treating with 10 per cent by volume of the reagents named, gave the results shown below. The rotation in each case is the average of three readings calculated to the undiluted basis:

| | |
|---|---|
| Basic lead acetate (official) | |
| (a) Lead not removed from filtrate | +0.7°V. |
| (b) Lead removed | −0.6 |
| Neutral lead acetate (solution containing 20 grams per 100 cc.) | |
| (a) Lead not removed from filtrate | +0.3 |
| (b) Lead removed | −0.7 |
| Alumina cream alone | −0.6 |

More recently, having had on hand some partly fermented apple juice with a nonvolatile acid content of 0.28 gram per 100 cc., the authors repeated Bender's experiments to see whether the same phenomenon would be observed with cider and, if so, whether it could be explained in the same way. The principal difference, of course, between the partly fermented juice and vinegar is that in the partly fermented juice there is more sugar and less acid present. This cider, when clarified with 10 per cent by volume of neutral lead acetate (solution containing 20 grams per 100 cc.) gave a reading of −3.2°V.; with the same volume of basic lead acetate (official), a reading of −3.5°V. When lead was removed from the filtrate both solutions gave a reading of −4.2°V. Thus there was observed the same shifting of the rotation as was noted with the vinegars previously mentioned.

Some observations were next made on the behavior of pure malic acid. Portions of a solution of malic acid which showed a rotation of −0.8°V. were treated with neutral lead acetate and basic lead acetate and these solutions then gave a reading of +1.1°V and +2.2°V., respectively. In the latter case, however, a considerable quantity of acetic acid was added to prevent precipitation. It was found that 5 to 6 per cent of acetic acid, the approximate quantity in a vinegar, was sufficient for this purpose. Bender's observations as to the behavior of malic acid under similar conditions were therefore confirmed.

The following experiments show, however, that in the case of ciders at least, on account of their low acidity as compared with that of vinegar, part of the nonvolatile acid is precipitated on the addition of both neutral and basic lead acetates. Portions of this cider, which contained 0.28 gram per 100 cc. of nonvolatile acid, were clarified with neutral and basic lead acetates and the lead subsequently removed from the filtrate with hydrogen sulfide. That portion clarified with neutral lead acetate then showed a nonvolatile acid content of 0.13

gram and the portion clarified with basic lead acetate a nonvolatile acid content of 0.09 gram per 100 cc.

The effect of fructose (levulose) is shown by the following experiment. A solution of fructose, which read −12.0°V., after the addition of neutral lead acetate read −11.9°V., and after the addition of basic lead acetate, −9.5°V.

When lead salts are used for clarification or decolorization the lactic acid present may also contribute to this shifting of rotation. A solution of lactic acid which showed a rotation of +3.9°V., on addition of neutral and basic lead acetates showed a rotation of +5.2°V. and +6.2°V., respectively. When the same experiment was repeated in 5 per cent acetic acid solution the rotations were +4.4°V. and +5.4°V., respectively.

The fact that the use of basic lead acetate is not permissible for the clarification of solutions containing levulose, when sugar is to be determined, is well known. It has also been known for some time that different salts have considerable effect upon the rotation of malic acid[1]. Dunbar and Bacon[1] in their work on the determination of malic acid showed the necessity of removing lead before polariscopic readings were made.

It is apparent from the foregoing discussion that the use of lead salts for the clarification of cider vinegar preliminary to polarization, particularly if lead is not removed from the filtrate, may lead to entirely misleading results. Since the principal, if not the only, use made of the polarization value of a cider vinegar, or, for that matter, of any vinegar, is as a criterion of purity, it is not apparent why clarification in the sense of removing other optically active substances than sugar should be necessary at all. What is wanted is the polarization value of the product under examination containing, in the case of genuine products, all of the substances, both in kind and quantity, which are normally present. This undoubtedly is what is meant by Bender when he used the term "true polarization" in his 1912 report. The ideal for this purpose would be the polarization value of the product without any treatment whatever. Such preliminary treatment as may be necessary with colored or turbid vinegars should be for the sole purpose of removing the turbidity and as much of the color as may be necessary to obtain a reading. For a number of years the Food Investigation Laboratory has been using "eponite" or "norit" for this purpose with entirely satisfactory results. It is believed that the method now prescribed by the association should be dropped and a method based upon the principles outlined in this paper adopted. The use of "eponite", "norit" or other similar decolorizing charcoals is recommended.

[1] *J. Ind. Eng. Chem.*, 1911, 3: 563; *Ibid.*, 826.

No report on flavoring extracts was made by the referee.

No report on meat and meat products was made by the referee.

No report on the separation of meat proteins was made by the associate referee.

No associate referee on the subject of the decomposition of meat products was appointed and no report on this subject was presented.

No report on gelatin was made by the associate referee.

No report on spices was made by the referee.

## SALAD DRESSINGS AND THEIR ANALYSIS.

By H. A. LEPPER (Bureau of Chemistry, Washington, D. C.).

The term "salad dressing" is a familiar one in probably every household yet these products have received little attention from food officials judging from recorded writings. In fact, no references to analyses of such products could be found in the literature. No standards for salad or mayonnaise dressings have been issued. Recipes and definitions given in cook books and dictionaries show the term "salad dressing" to be more general than "mayonnaise" which appears to be well understood as applying to a product made from egg yolk, a food oil and condiments. No recipe was found which called for the use of flour, gum, gelatin, starch or turmeric in mayonnaise dressing.

No systematic outline of methods for the analysis of salad dressings was to be found in the usual works on foods or food analysis. The following methods, therefore, were worked out for the analysis of these products:

### PREPARATION OF SAMPLE.

Mix the product until it is thoroughly homogeneous before sampling. This is especially necessary if the sample has stood for any appreciable time. Use approximately the quantity directed for the various determinations, noting accurately the actual weight. The weighing may be conveniently done from a Bailey weighing buret[1].

### TOTAL SOLIDS.

Weigh 10 grams of the sample into a tared lead dish (bottle cap) having a diameter of about 2½ inches and containing 10–15 grams of clean dry quartz sand. Evaporate on the steam bath to apparent dryness and then dry to constant weight in a vacuum oven at the temperature of boiling water. Cool in a desiccator and weigh. Weighings on samples high in solids should be made at 1-hour intervals. (Reserve the dry material for the determination of lecithin-phosphoric acid.)

[1] *C. A.*, 1916, **10**: 3003.

### ASH AND CHLORINE.

After drying 2 grams of the sample on the steam bath, determine the ash[1] and chlorine[2].

### REDUCING SUGARS BEFORE AND AFTER INVERSION.

Extract the oil from 20 grams of the sample in a wide-mouthed, 4-ounce bottle, by adding about 80 cc. of petroleum ether, shaking, and centrifugalizing. Draw off as much as possible of the petroleum ether solution (using suction and a short-stemmed pipet) and repeat the treatment with petroleum ether until all of the oil has been removed, as indicated by the absence of color in the solvent. Usually about four extractions are required.

Remove the petroleum ether from the residue with a current of air and transfer the residue with water to a 100 cc. graduated flask. Add 5–10 cc. of a fresh solution of metaphosphoric acid (prepared by dissolving 5 grams of the transparent lumps or sticks in cold water and making up to 100 cc.), mix thoroughly, make up to volume, and filter. Transfer 80 cc. of the filtrate, or as large an aliquot portion as possible, to a 100 cc. flask and, after neutralizing with a strong solution of sodium hydroxide, using phenolphthalein as indicator, cooling, and making up to the mark with water, determine the reducing sugar before inversion on an aliquot sample by the Munson and Walker method.

Invert another aliquot portion and determine the reducing sugar after inversion by the same method. Calculate as invert sugar in both cases.

NOTE.—Some dressings, particularly those containing starch, can not be clarified in this manner. It is then necessary to use the alcohol method for sugars[3]. When this method is used the residue from the petroleum ether extraction should be transferred to a 300 cc. flask with the 50% alcohol.

### SUCROSE.

Calculate the sucrose from the difference between the reducing sugar after inversion and before inversion.

### TOTAL ACID.

Titrate 10 grams of the sample in 400–500 cc. of recently boiled and cooled water with standard alkali, using phenolphthalein as indicator. Calculate as acetic acid.

### VOLATILE ACID.

Determine volatile acid on a 5-gram portion[4].

### NITROGEN.

Use 2–3 grams of the sample and determine nitrogen by the Kjeldahl-Gunning-Arnold method[5].

### OIL.

Determine the oil by the Roese-Gottlieb method[6] on 2 grams of the sample, using 2 cc. of concentrated ammonium hydroxide, 10 cc. of alcohol, and enough water to

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[1] *Assoc. Official Agr. Chemists, Methods.* 2nd ed., 1920, 71.
[2] *Ibid.*, 19.
[3] *Ibid.*, 94.
[4] *Ibid.*, 177, 25.
[5] *Ibid.*, 7.
[6] *Ibid.*, 227

fill the tube to just below the outlet, and making at least four extractions. Dry the oil in a vacuum oven at 70°C. to constant weight. (The residue remaining in the tube may be used for the turmeric test.)

### LECITHIN-PHOSPHORIC ACID.

Transfer the residue obtained in the determination of total solids to an extraction thimble adding also the lead dish which has been cut into pieces. Extract with absolute alcohol in an extractor of the siphon type in which the vapor heats the contents of the siphon thimble. After 10 hours' extraction, saponify the extract with alcoholic potassium hydroxide (for each gram of fat present use 5 cc. of a solution containing 8 grams of potassium hydroxide per 100 cc.) in a beaker or large platinum dish. Evaporate to dryness and ignite. Extract the charred mass with dilute nitric acid and filter. Return the paper to a dish and ignite to a white ash; dissolve in dilute nitric acid, filter and wash. Determine phosphoric acid in the united filtrates in the usual manner.

### STARCH.

Qualitative and quantitative determinations may be made by the methods given for meat products[1].

### GUMS.

Existing methods were found to be helpful in the identification of gums[2].

### BENZOIC ACID.

To determine benzoic acid or sodium benzoate, make 50 grams of the sample alkaline with strong sodium hydroxide. Extract the oil by shaking with petroleum ether. After the oil or fat is removed transfer the residue to a large evaporating dish and heat on the steam bath to coagulate the egg solids, adding alcohol to the extent of one-third the volume of the solution, if necessary. Transfer to a 300 cc. flask, saturate with salt, make up to the mark with saturated salt solution and filter. Extract benzoic acid from an aliquot portion of the filtrate and proceed as directed in the official quantitative method[3].

### TURMERIC.

Acidify with hydrochloric acid the residue remaining in the Roese-Gottlieb tube after the extraction of the oil and apply the filter paper boric acid test[4].

### ARTIFICIAL COLOR.

Make a portion of the sample alkaline with ammonium hydroxide and extract with petroleum ether. Test the extract if colored for oil-soluble colors, and the residue for other colors[5].

No attempt was made to identify the oil used. However, the residue obtained by evaporating the petroleum ether used to extract the oil in the sugar determination can be used for this purpose and the identity of the oil established by the usual chemical and physical tests.

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[1] *Assoc. Official Agr. Chemists, Methods.* 2nd ed., 1920, 212.
[2] *J. Ind. Eng. Chem.*, 1918, **10**: 530; *J. Am. Pharm. Assoc.*, 1920, **9**: 31.
[3] *Assoc. Official Agr. Chemists, Methods.* 2nd. ed., 1920, 120.
[4] *Ibid.*, 144
[5] *Ibid.*, 131.

A sample of mayonnaise was made in the laboratory from a typical recipe, as shown in Table 1. The results of the analysis of this sample and 10 others bought in the open market are given in Table 2.

Besides the result reported in the table for sucrose in the laboratory sample by the metaphosphoric acid method, a determination by the alcohol method for sugars was made and 0.07 per cent of invert sugar before inversion, 0.57 per cent of invert sugar after inversion and 0.48 per cent of sucrose was found. Sodium benzoate was added in the proportion of 0.05 gram to 50 grams of mayonnaise (0.1 per cent) and 0.055 gram or 0.11 per cent was found by the above method for benzoic acid. Juckenack[1] gives values for the lecithin-phosphoric acid content of eggs, stating that an average-sized egg yolk of 16 grams contains 0.1316 gram, or 0.82 per cent of lecithin-phosphoric acid. Calculation based upon these figures and the 0.107 per cent of lecithin-phosphoric acid found in the laboratory sample indicate the presence of about 13.0 per cent of egg yolk. This value does not agree very well with the 7.06 per cent actually used. While the percentage of lecithin-phosphoric acid, as determined by this method, may serve as an indication of the relative amounts of egg yolk in different samples, it is evident that more work must be done before these percentages can be used for more than a rough approximation of the actual quantity of egg yolk present.

TABLE 1.

*Composition of sample of mayonnaise prepared in the laboratory.*

| INGREDIENTS | WEIGHT | PERCENTAGE |
|---|---|---|
| | *grams.* | |
| Olive oil | 487 | 79.37 |
| Vinegar (cider) | 70 | 11.41 |
| Sugar | 2.99 | 0.49 |
| Salt | 6.64 | 1.08 |
| Mustard | 3.60 | 0.59 |
| Two egg yolks | 43.34 | 7.06 |
| Total.. ..... ..... | 613.6 | 100.0 |

The results show that the dressings on the market are of varying composition. The use of starch in two samples and gum tragacanth in one labeled as "mayonnaise" is shown. Judging from the well-understood meaning of the term "mayonnaise" the presence of these materials is an adulteration. Moreover, it can be noticed that in the sample containing 6.94 per cent of oil where gum tragacanth was found to be present there is a marked deficiency in oil content. This is also true of the samples containing starch. This would indicate a concealment of inferiority by the use of starch or gum. In one sample where starch is

[1] *Z. Nahr. Genussm.*, 1900, 3: 11.

TABLE 2.

*Results obtained on salad dressings.*

| SOLIDS | ASH | SALT | REDUCING SUGAR | | SUCROSE | ACID, AS ACETIC | | NITROGEN* | OIL | LECITHIN-PHOSPHORIC ACID | STARCH | | LABELED AS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Before inversion | After inversion | | Total | Volatile | | | | Qualitative | Quantitative | |
| *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | | *per cent* | |
| 86.26 | 1.19 | 1.09 | 0.06† | 0.61† | 0.52 | 0.56 | 0.51 | 0.207 | 83.16 | 0.107 | Negative | .... | ..... |
| 25.63 | 3.58 | 3.32 | 8.80‡ | 10.74‡ | 1.84 | 1.07 | 0.86 | 0.278 | 3.34 | 0.035 | Positive | 3.08 | Salad dressing |
| 56.02 | 2.74 | 2.40 | 1.72‡ | 1.78‡ | 0.06 | 1.03 | 0.75 | 0.318 | 46.93 | 0.085 | Negative | .... | Mayonnaise |
| 88.37 | 0.91 | 0.64 | 0.30† | 0.44† | 0.13 | 0.41 | 0.26 | 0.218 | 85.22 | 0.066 | Negative | .... | Mayonnaise |
| 88.73 | 0.59 | 0.30 | None† | None† | None | 0.41 | 0.29 | 0.261 | 86.02 | 0.081 | Negative | .... | Mayonnaise |
| 29.16 | 2.95 | 2.26 | 8.17‡ | 11.42‡ | 3.09 | 2.36 | 2.08 | 0.736 | 6.94 | 0.128 | Negative | .... | Salad (mayonnaise) dressing |
| 61.15 | 6.39 | 5.42 | 3.45‡ | 11.09‡ | 7.26 | 1.45 | 0.83 | 0.730 | 36.48 | 0.085 | Positive | 0.46 | Salad dressing a mayonnaise |
| 60.94 | 3.05 | 2.40 | None† | None† | None | 1.47 | 1.08 | 0.593 | 52.91 | 0.161 | Negative | .... | Salad mayonnaise dressing |
| 59.28 | 2.35 | 2.03 | None† | None† | None | 1.26 | 1.06 | 0.468 | 52.83 | 0.095 | Negative | .... | Mayonnaise |
| 36.36 | 3.15 | 2.73 | 3.24‡ | 12.31‡ | 8.62 | 1.89 | 1.60 | 0.374 | 12.52 | 0.106 | Positive | 4.42 | Mayonnaise |
| 62.33 | 3.13 | 2.65 | 0.07† | 0.07† | None | 1.18 | 0.89 | 0.547 | 54.68 | 0.145 | Negative | .... | Salad dressing |

*Determinations made by the Nitrogen Laboratory of the Bureau of Chemistry.

†Metaphosphoric acid method.

‡Alcohol method.

used, the oil content was found to be 36.48 per cent which is low but not excessively so. Another sample, sold as salad dressing, was labeled, in part, "Ingredients sugar, cereal, salt, mustard, egg, vinegar, oil, certified color". It may be noticed in this case that the use of cereal has allowed as little as 3.34 per cent of oil to be used. This sample was not labeled "mayonnaise" and the presence of cereal was declared. Naphthol Yellow S was detected in this sample. All the samples were tested for turmeric and benzoic acid with negative results.

It is recognized that the methods herein suggested are open to improvement and that there is much opportunity for further work on the analysis of salad dressings.

## REPORT ON THE DETERMINATION OF SHELLS IN CACAO PRODUCTS.

By W. C. TABER[1] (U. S. Food and Drug Inspection Station, Park Avenue Building, Baltimore, Md.), *Referee.*

The shell of the cocoa bean amounts to about 11 to 13 per cent of the total weight of the bean. The greater part of this shell is removed from the bean in the process of manufacture of chocolate goods, but there always remains a small residuum which it is impossible to separate from the nibs by any factory machinery. This small residue of shells in the nibs may be increased by faulty operation in the factory, either purposely or through careless management. Shells also may be added to the liquor by the deliberate addition of the "fines" which is the finest particles of nibs and shells that come from the fanning machine and usually is about one-half shell. The estimation of these excessive amounts is the problem of the inspection chemist.

Many different physical and chemical methods have been proposed for the determination of shell, particularly by the German chemists. Of the former methods that of Filsinger[2] by a sedimentation process, that of Macara[3], and that of Goske[4] of flotation in calcium chloride solution, have all been tried by various investigators and found lacking. The variations in the fineness of grinding and in the specific gravity of the shell particles are serious defects in the quantitative results of these processes.

Of the chemical methods which have been suggested in recent years. the determination of pentosans was emphasized by Adan[5]; the determination of cocoa red, which Ulrich[6], the author, states will show only

[1] Present address, U. S. Food and Drug Inspection Station, Federal Building, Buffalo, N. Y.

[2] *Z. öffent. Chem.*, 1899, 5: 27

[3] E. R. Bolton and Cecil Revis. Fatty Foods. Their Practical Examination. A Handbook for the Use of Analytical and Technical Chemists. 304.

[4] *Analyst*, 1910, 33: 162.

[5] Seventh Intern. Cong. Appl. Chem., 1909, VIII C: 194.

[6] *Arch. Pharm.*, 1911, 249: 524.

10 per cent or more of shells; more recently Keller[1] proposes the color of the ether extract as a method, that from shell being darker than extract from the nibs. A very good resumé of these and other methods has been given by Ulrich and also by Knapp and McLellan[2].

Beythien and Pannwitz[3] state that the total and water-soluble phosphoric acid are means of determining the amount of shells present. They conclude, however, as do Knapp and McLellan and other investigators, that the crude fiber is the most important single determination that can be made. Since this has been so generally agreed upon, it is well to look at the data available on the crude fiber determination to see what results may be expected.

Consideration first must be given to the fact that clean nibs free from shells give a certain figure for the crude fiber, varying with the different varieties of the beans, the temperature of the roasting, the fineness of the grinding and, Knapp and McLellan state, with the ripeness of the bean and the degree of fermentation. The soft pulp that surrounds the bean is decomposed by fermentation, and so runs off from the mass of beans as a liquor. If the fermentation is not carried sufficiently far some of this pulp remains and hardens on the beans so that it can not be separated from the shell. Data obtained in the Bureau of Chemistry by B. H. Silberberg and the referee show that sometimes this dry pulp remaining on the beans increases the apparent weight of the shell by 2 per cent or more.

The following table shows the maximum, minimum and average figures obtained for crude fiber by various investigators, both in the clean nibs and on the shells from various varieties of beans.

TABLE 1.

*Crude fiber on dry fat-free material.*

| INVESTIGATOR | ROASTED NIB | AVERAGE | ROASTED SHELL | AVERAGE |
|---|---|---|---|---|
| | *per cent* | *per cent* | *per cent* | *per cent* |
| Booth, Cribb and Richards* | 4.7—6.2 | .... | 13.2—16.3 | .... |
| Winton, Silverman and Bailey† | 4.7—6.6 | 5.6‡ | 13.7—20.7 | 18.0‡ |
| Knapp and McLellan§ | 4.6—6.8 | 5.8** | 15.4—21.4 | 18.7** |
| Bureau of Chemistry†† | 5.5—7.7 | 6.2‡‡ | 14.7—24.4 | 18.6‡‡ |
| W. C. Taber and M. L. Offutt§§ | 5.9—7.5 | 6.5*** | 14.8—23.9 | 18.7*** |

* *Analyst*, 1909, 34: 134.
† Rept. Conn. Agr. Exp. Sta. 1902, 248.
‡ Average results of analyses of 17 varieties of beans.
§ *Analyst*, 1919, 44: 2.
** Average of 10 determinations of 8 varieties of beans
†† U. S. Bur. Chem. Information from Eugene Bloomberg and W. C. Taber.
‡‡ Average of 21 determinations of 21 varieties of beans.
§§ Unpublished, 1920 data.
*** Average of 12 determinations of 7 varieties of beans.

[1] *Arch. Pharm.*, 1917, 255: 405.
[2] *Analyst*, 1919, 44: 2.
[3] *Z. Nahr Genussm.*, 1916, 31: 265.

It is interesting to note in Table 1 the quite close agreement obtained by the different workers in recent years, the average being in especially close agreement. It is worthy of note, however, that the analysts in the Bureau of Chemistry have obtained somewhat greater extremes of crude fiber both in the case of the nibs and also of the shells. This may be due in part to the kind of beans used recently. It has come to the writer's attention that about one-half of the beans imported into this country are of the Accra variety. From Table 2 it is seen that the fiber on the Accra nibs and shells runs higher than most other varieties. This seems to be particularly apparent in the figures for shells.

Table 2 gives in detail the figures by Taber and Offutt quoted under Table 1.

TABLE 2.

*Crude fiber on moisture and fat-free basis.*

| VARIETY OF BEAN | NIBS | SHELLS |
|---|---|---|
| | *per cent* | *per cent* |
| Accra | 7.47 | 22.77 |
| Accra | 6.76 | 23.90 |
| Arriba | 6.85 | 17.33 |
| Bahia | 6.14 | 20.18 |
| Bahia | 5.97 | 18.93 |
| Caracas | 5.82 | 16.82 |
| Caracas | 6.88 | 15.22 |
| Sanchez | 6.22 | 18.62 |
| Sanchez | 5.87 | 19.71 |
| Trinidad | 7.13 | 16.64 |
| Trinidad | 5.88 | 14.79 |
| Machala | 7.44 | 20.05 |
| Average. ... | 6.54 | 18.74 |

These figures represent beans obtained during the summer of 1919 by B. H. Silberberg and the writer at different chocolate factories in the east, and roasted in the laboratory at a temperature of 140°C. The figures verify the statement previously made that even the same variety of bean may vary in the fiber content on account of the degree of ripeness, and other factors.

In the information card by Eugene Bloomberg and the writer, cited in Table 1, it was stated that since the crude fiber on only one sample of beans ran above 7 per cent, it seemed safe to assume that figure as a maximum for the purpose of calculating the amount of shell present in any chocolate sample. It will be noted from Table 3, however, that three varieties ran somewhat above 7 per cent, and one of them was a variety largely used. It is possible, therefore, that further investigation will warrant raising this maximum figure somewhat.

From Table 1 it would appear that the figure for fiber in shells might well be taken at about 19 per cent for the purpose of calculating the

results. This would indicate that an increase of 1 per cent in fiber above the standard for the nibs would mean an increase of shell content of approximately 8 per cent. In order to determine how closely this calculation could be applied, mixtures of defatted ground shell and nibs passing through a 100-mesh sieve were prepared in different proportions and crude fiber determined on this mixture. The results are given in Table 3.

TABLE 3.

*Crude fiber on mixture of defatted ground shell and nibs.*

(Analyst, M. L. Offutt.)

| SAMPLE NUMBER | SHELL PRESENT | CRUDE FIBER FOUND |
|---|---|---|
| | *per cent* | *per cent* |
| 1 | 2.50 | 4.60 |
| 2 | 3.50 | 6.77 |
| 3 | 4.00 | 8.08 |
| 4 | 4.80 | 7.38 |
| 5 | 6.00 | 7.26 |
| 6 | 6.00 | 8.70 |
| 7 | 6.50 | 8.85 |
| 8 | 7.00 | 9.25 |
| 9 | 7.00 | 9.01 |

It should be stated in connection with Table 3 that Samples 2, 3, 5, 8 and 9 were composed of the same varieties of nibs and shells, and were different from the varieties found in the other samples. This would account for some of the differences that are apparent, for instance in Nos. 5 and 6, with the same amount of shell but with quite large variation in fiber. The fiber content in Nos. 6, 7, 8 and 9 would indicate a much larger percentage of shells than is actually present if the figures for nibs and shells indicated above are used. This is in accordance with the experience of Knapp and McLellan who would deduct 8 per cent from the shell content, as found by the fiber method, in order to determine the actual amount present. It is apparent that in the samples just referred to this would give a close approximation to correct results. It is also evident from Table 3 that the detection of small amounts of shells is impossible by the crude fiber method. It apparently is necessary to have about 4 per cent of shells in the sample before excessive amounts of fiber are indicated.

This also can be made clear from mathematical consideration. Taking a mixture of 5 per cent of shells with a fiber content of 18.7 per cent, and nibs with a fiber content of 6.5 per cent, the resultant mixture would yield a fiber content of 7.1 per cent, which is only slightly above the maximum. With a mixture of 2 per cent of shells with the same fiber content, the resultant mixture would yield a fiber content of 6.8 per cent, which is within the maximum 7 per cent limit.

Knapp and McLellan give a range of from 5.3 to 6.8 per cent on a moisture fat-free basis in the determination of crude fiber on pure cocoa samples sent to different chocolate laboratories in England. The writer is unable to account for this wide variation, as in his experience duplicate determinations agree within one-tenth of 1 per cent, particularly with material within that range of fiber content.

It seems, therefore, that collaborative work might well be done on this determination during the coming year. The consensus of opinion seems to be that this is the best single chemical determination even though it does not show the presence of small amount of shells.

Fortunately, there is another method which can be used in the determination of the shell content of cacao products. The microscope has given great promise of usefulness in this field. This method is based on the presence of various characteristic tissue found in the shell of the cocoa bean, such as the spongy tissue, stone cells, spiral vessels, mucilage cells, etc. Hanausek[1] states that the mucilage cells alone are characteristic. Beythien and Pannwitz[2] make use of the mucilage cells as well as the stone cells. They state that if more than 6 mucilage cells with a magnification of 90 are found when 4 or 5 mg. of fat-free chocolate are used, the cocoa is adulterated with more than 5 per cent of shells. The writer's work would indicate that about 6 mucilage cells are found in 4 mg. of fat-free chocolate containing only 2 per cent of shells, and in 5 per cent of shells there would be at least 15 mucilage cells.

Boericke[3] makes use of the stone cells as a criterion of shell content, but does not make a complete count of the number found in any given quantity of material.

Collin[4] makes use of very fine sieves for separating the insoluble fiber from the starchy material and, after decolorizing the residue, examines it for microscopic characteristics, the microscope apparently being used for qualitative results alone.

Various analysts have used quite different procedures for preparing the chocolate material for examination, but this subject can not be discussed in detail. Several analysts in the Bureau of Chemistry have used the method of counting stone cells in a given quantity of material for some years past. The procedure is as follows:

Transfer 5–10 grams of the chocolate or cocoa to a centrifugal bottle, and treat with successive portions of gasoline or ether, centrifugalizing thoroughly each time until the fat is removed. Then wash the sample with water in the same way to remove sugar. After the sugar has been removed, finally wash the sample with alcohol and ether, dry, and mix thoroughly. Weigh accurately 2 mg. of the sample on a glass slide, add 1–2 drops of 60% chloral hydrate solution, mix thoroughly by stirring with the point of a needle, cover with a cover glass and allow to stand until the tissues have

[1] *Apoth. Ztg.*, 1915, **30**: 590.
[2] *Z. Nahr. Genussm.*, 1916, **31**: 265.
[3] *Pharm. Zentralhalle*, 1916, **57**: 283.
[4] *J. pharm. chim.*, 1910, 7th ser., **1**: 329.

cleared. In case haste is necessary, the clearing may be obtained by gentle heating. Examine the entire amount, counting all the stone cell groups, and compare with a mount, prepared in the same way from a standard sample containing a known percentage of cocoa shells.

The accuracy of the method has not been thoroughly tested by collaborative work. Known samples were sent to a few analysts who had had experience in the use of the method, but, unfortunately, the returns have not been sufficient to draw any definite conclusions. It would seem, however, that with a maximum of 5 per cent of shells on the fat-free basis, fairly accurate results may be secured. When larger amounts of shells are present, the error appears greater. This is not so serious a matter, however, since goods manufactured by reputable manufacturers and under good factory conditions will contain considerably less than 5 per cent of shells on the fat-free basis. The percentages found on the fat-free basis will necessarily be divided by 2 to estimate them on the original chocolate liquor, which contains approximately 50 per cent of fat. Thus the error of examination is divided by 2.

The disadvantages of this method are that it is extremely trying and tedious, requiring considerable experience in the absolute identification of some of the stone cells on account of the thick tissue often encountered. The statement of Knapp and McLellan that they found no procedure which would determine as low a percentage of shell as 5, is quite contrary to the experience of the writer and others in the Bureau of Chemistry. They tried the microscopic method, but evidently did not succeed in making it quantitative. The counting of the mucilage cells is apparently a less accurate gauge of the amount of shell tissue present, since the number of these cells found for a given amount of material is considerably less than the number of stone cells. It has the advantage that these cells are large and comparatively easy to distinguish.

In comparing results by the microscopic method with those obtained by the crude fiber determination, one sometimes meets conflicting data, as shown in Table 4.

TABLE 4.

*Moisture and fat-free basis.*

| SAMPLE NUMBER | CRUDE FIBER* | SHELL MICROSCOPIC EXAMINATION† |
|---|---|---|
| | *per cent* | *per cent* |
| 1 | 5.26 | 2 |
| 2 | 6.95 | 2 |
| 3 | 7.70 | 4–5 |
| 4 | 6.23 | 4 |
| 5 | 6.67 | 10 |
| 6 | 7.01 | 3 |
| 7 | 8.13 | 10–12 |
| 8 | 6.59 | 12–14 |
| 9 | 6.55 | 20 |

*Analyst, M. L. Offutt.
†Analyst, B. H. Silberberg.

## RECOMMENDATIONS.

It is recommended—

(1) That further study be made of the microscopic method for the examination of cacao products for shells in order that its limit of accuracy may be determined by experienced microscopists.

(2) That further study be made of the chemical methods for shells, particularly crude fiber, with a view to obtaining more data for the interpretation of results, and for the purpose of correlating chemical results with microscopical results.

It is the opinion of the writer that the present method of stating the permissible amounts of ash, fiber, and starch is unscientific and results in some confusion. It would be simpler and the figures could then be much more easily applied to all cacao products if these constituents were stated on the fat and moisture-free basis. To illustrate, paragraph 6 of the standards[1] reads as follows:

> Sweet chocolate, sweet chocolate coatings, contains in the sugar and fat-free residue no higher percentage of ash, fiber, or starch than is found in the sugar and fat-free residue of chocolate.

The standards do not state the percentage of ash, fiber, and starch found in the sugar and fat-free residue of chocolate. Of course such figures may be obtained by calculating from paragraph 4, which contains the standards for chocolate, chocolate liquor, etc., but in that case an error is incorporated if it is assumed that the chocolate has 45 per cent of fat, when it contains, as a matter of fact, more than 50 per cent of fat.

The same error enters again in the standard for cocoa and sweetened cocoa in which it is necessary to correct for the fat removed. It is seen, therefore, that the error of a 45 per cent fat basis enters into paragraphs 4, 6, 8 and 10. It would, therefore, be more exact and more scientific to express the standards of all kinds of cacao products on a fat, moisture, and sugar-free basis.

It is, therefore, recommended—

(3) That a revision of the standards for cacao products along the lines indicated be recommended to the Committee on Cooperation with Other Committees on Food Definitions.

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[1] U. S. Dept. Agr., Office of the Secretary, Circ. 136: (1919), 18.

# CACAO PRODUCTS WITH SPECIAL REFERENCE TO SHELL CONTENT.

By B. H. SILBERBERG (Bureau of Chemistry, Washington, D. C.).

For some time the question of the allowable amount of cocoa shell in cocoa and chocolate products has been discussed in the Bureau of Chemistry. Principally for the purpose of getting some information on this subject, W. C. Taber and the writer visited a number of factories in June and July, 1919, and samples were collected for investigation.

It may be advisable first to describe briefly the different stages in the process of manufacture of these goods in order to show at what points quantities of shell may enter in excess of that which is easily preventable in good commercial practice. The beans arrive in this country in large sacks and are stored in warehouses until needed in the factory. When taken to the factory they first undergo a cleaning process to remove dirt and stones and other extraneous matter. They are then placed in a roaster, which is generally of the revolving type, where the beans are carefully roasted for about one-half hour. After the roasting is completed they are drawn out into trays with perforated bottoms where they are cooled quickly in order to prevent sweating, and also to expedite the factory work. This cooling is often accomplished by suction or cold air blasts.

The size of beans and thickness of the shell are both factors influencing the percentage by weight of shell to nib found in any given variety of bean. Fifteen varieties of raw beans commonly used at the present time, some clayed and some unclayed, were examined in order to determine their percentage of shell. Of twenty-four samples of unclayed beans, including fifteen varieties, the highest shell content found was 15.6 per cent, the lowest 7.3 per cent, and the average 12.1 per cent. Several of those which were highest in shell had an unusual amount of pulpy matter from the pod dried on them, a few contained a large amount of shriveled beans of poor quality, and the beans in most of these samples were somewhat under average size. Four samples of clayed beans of three different varieties showed a maximum shell content of 17.3 per cent, a minimum of 13.5 per cent and an average of 15.5 per cent.

After the roasted beans have been cooled they are cracked and fanned to remove the shell. These operations are both accomplished in one machine, the cracker consisting of rolls between which the beans are broken to loosen the shell from the nibs. After this cracking process, the lighter shell particles are separated from the heavier chocolate nibs by air blasts which also separate the nibs of different gravity, the lighter shell particles being removed at one end of the machine together with

the lighter particles of broken nibs. Shell particles, which are unusually heavy, due to claying or perhaps to attached tissue, will naturally come through with the nibs. Whole beans which are shriveled or too small to have been caught between the cracking rolls may come through with the larger nibs. These are the factors which influence to the largest extent the shell content in the final product. On account of the variation in the size of beans and in the weight of the shells in different varieties of beans, it is customary to crack and fan each variety separately in order that the rolls may be set and the blast adjusted accordingly. The nibs of different varieties are usually blended before grinding.

The germs of the beans usually come through with the small-sized nibs and, in most factories, are removed by running the nibs over vibrating planes which are perforated to allow the small germ to pass through without any great loss of nibs. The percentage of germ in twenty-eight samples of fifteen different varieties of beans showed a maximum of 0.9 per cent, a minimum of 0.5 per cent and an average of 0.73 per cent.

The last or lightest portion containing some very small particles of nibs and a considerable quantity of shell particles carried over by the blast is usually discarded in the best factories. This final separation, sometimes called "fines" or "dust", usually consists of 50 per cent or more of shell. In some factories efforts are made to clean the fines to recover some of the nibs. As a rule this has not appeared to be satisfactory.

In the different separations made by the fanner the percentage of shell is nearly always much higher in the finest separation, running as high as 10 per cent and over in some of these last divisions. It should be emphasized, however, that a rather small proportion by weight of these last separations is used in the final product.

The nibs after leaving the fanner are blended for the purpose of obtaining a desirable flavor, the stronger flavored beans being used with some of milder flavor. It is usual to make a blend of two or three varieties of beans, although sometimes more are used. The nibs are placed in a hopper which feeds directly into the mills composed of two or three sets of revolving stones. The ground nibs come from these stones in the form of a heavy viscous mass, known to the trade as "chocolate liquor". This liquor is the material from which all finished goods are made. In some cases the liquor is run into presses where more than half of the cocoa butter is extracted. The residual press cake is then ground and run through bolting cloth and is the cocoa of commerce. There seems to be no uniformity in the size of bolting cloth used.

The liquor from which fat is not extracted is further refined for coatings, sweet chocolate or milk chocolates. This refining consists in

further grinding and working in melangeurs, steel rolls, and longitudinals to make the goods smoother and more palatable. During this process cocoa butter, sugar, or milk may be added, according to the finished product desired. After this refining is completed the chocolate is often allowed to stand in a warm room for some days to "season" or develop flavor before it is run into molds.

Samples of nibs were collected from the spouts of the hoppers as they fed into the grinding mills in order to have cleaned nibs which represented the liquor used in the final product. In each of these samples, which in most cases were blends of several varieties of beans, the percentage of shell was determined by hand separation with the following results:

PERCENTAGE OF SHELL IN NIBS FROM HOPPERS.

Eleven factories out of seventeen showed an average of 1.32 per cent, a maximum of 2.55 per cent, and a minimum of 0.65 per cent of shell. Fifteen factories, including the eleven just mentioned, showed an average of 1.72 per cent, a maximum of 3.65 per cent, and a minimum of 0.65 per cent of shell. Seventeen factories, the total number visited, showed an average of 2.24 per cent of shell.

It may be seen that with the exception of two factories in which the shell content in the nibs was evidently excessive, the average percentage of shell in nibs in fifteen factories was below 2 per cent. Although nibs from six of those factories contained shell in excess of 2 per cent it is believed that for the most part this was due to the use of fines and to careless and uncleanly factory practices, and in one case was partly due to disturbed factory conditions incident to the installation of new machinery. Another important factor which influenced the percentage by weight of shell in these nibs was the presence in at least three cases of clayed beans in the blend. While this makes the shell content, as determined in this way, appear excessive, the actual amount of shell tissue, minus the clay, as determined by chemical and microscopical methods, would be less than indicated by weight. This clay, which here figures as weight of shell, would appear in the chemical analysis as acid-insoluble ash and would not figure at all as shell in the microscopical examination.

It may also be seen that of these fifteen factories the nibs from five, or 33 per cent, contained 1 per cent or under of shell, while eight, or 53 per cent, had 1.5 per cent or less of shell.

From these results it seems reasonable to conclude that a fair limit of tolerance for shell in cocoa or chocolate products would be 2 per cent on the basis of the original nibs or liquor, or 4 per cent on a fat and sugar-free basis.

# REPORT ON METHODS FOR THE EXAMINATION OF CACAO BUTTER.

By W. F. BAUGHMAN (Bureau of Chemistry, Washington, D. C.), *Referee.*

The last report of a referee on cacao butter was made by Eugene Bloomberg at the 1916 meeting of the association[1]. His report was concerned with the critical temperature of dissolution determination and a test for tallow and hydrogenated oil which he had originated. The committee on recommendations of referees recommended that these two methods be further studied. Your referee thought it advisable, therefore, to make a critical examination of these two methods before again submitting them to collaborators. After the conclusion of this examination, there was not sufficient time to send out samples to collaborators. So the present report contains no results of collaborative work, but it is hoped that the investigation conducted by the referee has cleared up some obscure points and made the methods more reliable and workable.

The critical temperature of dissolution determination is practically the Valenta test[2]. Cacao butter and other fats dissolve in acetic acid on heating. The critical temperature of dissolution is the temperature at which a solution of 5 cc. of melted fat in 5 cc. of glacial acetic acid becomes turbid on cooling. Practically all potential substitutes for cacao butter with the notable exceptions of hydrogenated oils, oleostearine and tallow, have a considerably lower temperature of dissolution than cacao butter, and when mixed with pure cacao butter they lower the critical temperature by an amount approximately proportional to the amount substituted. The critical temperature of dissolution of any fat varies with the strength of the acetic acid, and Bloomberg therefore recommends that the acetic acid be standardized against an authentic sample of cacao butter. The purity or sophistication of the sample under examination is indicated by comparing its critical temperature with that of the authentic cacao butter.

Bloomberg sent six samples of adulterated cacao butter to five collaborators. They made a critical temperature of dissolution determination on each sample. Four reported adulteration of each sample. The fifth drew no conclusions from his examinations, but his results plainly indicated adulteration.

It is obvious that the reliability of this method depends on the constancy of the critical temperature of dissolution of cacao butter produced under different conditions of manufacture, or from beans grown in different localities. The results in Bloomberg's report do not shed

[1] *J. Assoc. Official Agr. Chemists*, 1920, 3: 486.
[2] *J. Soc. Chem. Ind.*, 1884, 3: 643.

TABLE 1.

*Critical temperature of dissolution of cacao butter and substitutes.*

| SAMPLE NUMBER | DESCRIPTION OF SAMPLE | ACID VALUE | TEMPERATURE OF DISSOLUTION | |
|---|---|---|---|---|
| | | | Observed | Corrected |
| | | | °C. | °C. |
| 1 | Cacao butter, Standard | 1.07 | 90. | 91.3 |
| 2 | Cacao butter, Trinidad | 1.22 | 89.5 | 91.5 |
| 3 | Cacao butter, Sanchez | 1.08 | 90.5 | 91.8 |
| 4 | Cacao butter, Caracas | 1.12 | 90.5 | 91.8 |
| 5 | Cacao butter, Bahai | 1.12 | 89.5 | 90.8 |
| 6 | Cacao butter, Accra | 1.55 | 90. | 91.8 |
| 7 | Cacao butter, commercial sample | 3.51 | 86. | 90.2 |
| 8 | Cacao butter, commercial sample | 2.86 | 86. | 89.2 |
| 9 | Cacao butter, commercial sample | 2.55 | 86.5 | 89.6 |
| 10 | Cacao butter, commercial sample | 2.97 | 87. | 90.6 |
| 11 | Coconut oil stearine | .... | Soluble at room temperature | .... |
| 12 | Palm kernel oil stearine | .... | Soluble at room temperature | .... |
| 13 | Cottonseed oil | .... | 52.5 | .... |
| 14 | Cottonseed oil (wintered) | 0.30 | 43. | .... |
| 15 | Cottonseed oil stearine | .... | 60.5 | .... |
| 16 | Sesame oil | .... | 52. | .... |
| 17 | Peanut oil | 1.00 | 65. | .... |
| 18 | Hydrogenated cottonseed oil | .... | 98.5 | .... |
| 19 | Tallow | .... | 85. | .... |
| 20 | Oleo stearine | .... | 91. | .... |
| 21 | Cacao butter containing 5% coconut oil stearine | .... | 85. | .... |
| 22 | Cacao butter containing 10% coconut oil stearine | .... | 82.5 | .... |
| 23 | Cacao butter containing 20% coconut oil stearine | .... | 73. | .... |
| 24 | Cacao butter containing 5% palm kernel oil stearine | .... | 87. | .... |
| 25 | Cacao butter containing 10% palm kernel oil stearine | .... | 82.5 | .... |
| 26 | Cacao butter containing 20% palm kernel oil stearine | .... | 74. | .... |
| 27 | Cacao butter containing 5% cottonseed oil | .... | 87.5 | .... |
| 28 | Cacao butter containing 10% cottonseed oil | .... | 86. | .... |
| 29 | Cacao butter containing 20% cottonseed oil | .... | 83. | .... |
| 30 | Cacao butter containing 10% cottonseed oil stearine | .... | 87. | .... |
| 31 | Cacao butter containing 20% cottonseed oil stearine | .... | 83. | .... |
| 32 | Cacao butter containing 30% cottonseed oil stearine | .... | 80. | .... |
| 33 | Cacao butter containing 10% peanut oil | .... | 86.5 | .... |
| 34 | Cacao butter containing 20% peanut oil | .... | 84. | .... |
| 35 | Cacao butter containing 30% peanut oil | .... | 82. | .... |

much light on this point. Accordingly, the critical temperature of dissolution has been determined on six samples known to be pure and four commercial samples supposed to be pure. The results are given in Table 1. Samples 2 to 6, inclusive, were pressed in a commercial

TABLE 2.

*Influence of acidity of cacao butter on the critical temperature of dissolution in glacial acetic acid.*

| Sample Number | DESCRIPTION OF SAMPLE | ACID VALUE | DISSOLUTION TEMPERATURE | FALL IN DISSOLUTION TEMPERATURE PER UNIT OF ACID VALUE |
|---|---|---|---|---|
| | | | °C. | °C. |
| 1 | Cacao butter (standard) | 1.07 | 89. | .... |
| 2 | Cacao butter to which has been added cacao butter fatty acids | 3.22 | 86. | 1.39 |
| 3 | Cacao butter to which has been added cacao butter fatty acids | 5.92 | 83. | 1.29 |
| 4 | Cacao butter to which has been added cacao butter fatty acids | 11.12 | 78. | 1.09 |
| 5 | Cacao butter to which has been added cacao butter fatty acids | 19.68 | 67. | 1.18 |

plant in the presence of H. S. Bailey, formerly of the Bureau of Chemistry, from beans grown in various localities. They represent, therefore, butters of commercial grade. Samples 7 to 10, inclusive, were collected in the open market by W. C. Taber. The observed critical temperatures of the first six samples are practically constant, but the results obtained on the other four samples are lower and the variations are wide enough to cause one to be suspicious of their purity. However, the acid values of the four commercial samples are greater than the acid values of the six authentic samples.

It is well known that the acidity of a fat influences the results of the Valenta test. Free acids in cacao butter have a similar influence on the critical temperature of dissolution. The influence is illustrated by the results given in Table 2.

Samples 2 to 5, Table 2, were prepared by adding portions of free fatty acids obtained from pure cacao butter to the butter represented by Sample 1. The third column in that table gives the acid values (mg. of potassium hydroxide required to neutralize the free fatty acids in 1 gram of the sample). In the fourth column are tabulated the observed critical temperatures, and in the fifth column is given the lowering of critical temperature per unit of acid value. The lowering is proportional to the acid value. One unit of acid value causes an average reduction of 1.2° C. If this factor is used to correct the results obtained on the commercial butters (Samples 7 to 10, Table 1) it is found that the corrected results are in line with those for the authentic samples. It is, therefore, important to determine the acidity and to make the proper correction if necessary.

The critical temperatures of dissolution of some of the possible adulterants of cacao butter have been determined (Samples 11 to 20). With

the exception of hydrogenated cottonseed oil, tallow and oleo stearine, they are all considerably lower than cacao butter.

The results obtained on Samples 21 to 35 show to what extent adulteration with these various products lowers the critical temperature of dissolution. The results indicate that one should, by the use of this method, be able to detect 5 per cent or more of coconut or palm kernel oil stearine. Peanut oil, cottonseed oil and cottonseed oil stearine have higher critical temperatures than coconut or palm kernel oil stearine, and when mixed with cacao butter, the critical temperature of the latter is reduced to a less extent. Perhaps one can not detect with certainty adulteration with less than 10 per cent of these products.

The apparatus and details of the method used by the author in making the determination may be of interest. A thermometer is inserted tightly into a cork fitting a 6×¾ inch test tube, a small groove being cut in the side of the cork for the escape of hot air. The thermometer extends down far enough to be covered completely by 10 cc. of liquid. Graduation marks are scratched on the test tube at 5 cc. and 10 cc. from the bottom. The melted fat is poured into the tube up to the 5 cc. mark and then acetic acid up to the 10 cc. mark. The cork holding the thermometer is inserted and the test tube is placed in a larger one (4½×1½ inches) containing glycerol, and held firmly in place with a cork. Heat is applied and the apparatus frequently shaken until a clear solution of fat in acetic acid is obtained. The solution is then allowed to cool with constant shaking, without removing it from the glycerol bath, and the temperature noted at which it becomes turbid. By not removing the solution from the glycerol bath, it cools more slowly, and permits the dissolution temperature to be read more sharply and accurately. The fat and acid should be measured very carefully as small variations in the proportions of fat and acid affect the results. The fat should be filtered through filter paper in a hot air oven (100°C.) in order to remove traces of moisture. It is then well to allow it to cool somewhat before measuring the 5 cc. sample and to measure the sample and standard butter at the same temperature. The acetic acid used was labeled "Acid Acetic, Glacial, contains 99.5% of absolute acetic acid."

### TEST FOR HYDROGENATED OIL, TALLOW, OLEOSTEARINE, LARD AND PARAFFIN.

Bloomberg's directions for making the acetone test for hydrogenated oils and tallow are to dissolve 5 cc. of melted fat in 5 cc. of acetone, heating if necessary, and to allow the mixture to stand overnight in cold water. If tallow or hydrogenated oil is present, a flocculent precipitate is obtained. It is the opinion of the referee that these directions are too indefinite. If the water is very cold, cacao butter

will solidify and the analyst might confuse this with the precipitate caused by tallow or hydrogenated oil. Indeed, one of Bloomberg's collaborators had this experience and reported the presence of an adulterant in a sample of pure cacao butter. If the room is not too warm (say at 20° to 22°C.) the solution of fat in acetone may be allowed to stand overnight at room temperature. If hydrogenated oil, tallow, oleostearine, lard, or paraffin is present, a precipitate is formed, while pure cacao butter will not solidify or precipitate.

In order to shorten the time required for the test, Bloomberg suggests using a mixture of equal parts of acetone and carbon tetrachloride instead of acetone. Since fats are more soluble in this mixture, it is necessary to cool the solution in ice water for 5 to 30 minutes. A flocculent precipitate is obtained if hydrogenated oil, tallow, oleostearine, lard or paraffin is present. A blank should be run using pure cacao butter. Sometimes a precipitate is obtained in a sample of pure butter, so if the sample being tested gives a precipitate, it should be removed from the ice water and allowed to remain at room temperature for a time. If the precipitate is only solidified cacao butter, it will soon melt and go into solution; if the precipitate is due to any of the above-mentioned fats, a much longer time will be required for it to go into solution. Less than 5 per cent of these substitutes can be detected by this method. The referee regards the acetone-carbon tetrachloride mixture as preferable.

### RECOMMENDATIONS.

It is recommended—

(1) That further collaborative work be done on the critical temperature of dissolution determination, especially to test the accuracy of the correction factor for acidity.

(2) That the test for tallow, hydrogenated oils, lard, paraffin, etc., be further studied, since the acetone-carbon tetrachloride test has never been tested by collaborators.

## REPORT ON COFFEE.

By H. A. LEPPER (Bureau of Chemistry, Washington, D. C.), *Referee.*

The determination of caffeine in coffee has held the attention of the association continuously since 1908 with the exception of 1912, when no report was made on coffee, and 1916, when experimental work on the moisture content and the chemical composition of raw and roasted coffees was reported and no collaborative work was undertaken.

When the present referee was appointed in 1917, the Stahlschmidt method for caffeine in coffee had been provisionally adopted two years

previously[1] and its further study with a view to official adoption in 1917 was recommended. In the study of the Stahlschmidt method in 1917, comparison was made with the Fendler-Stüber method[2]. The advantages of the latter were such that it was adopted as a tentative method and no further action was taken on the Stahlschmidt method.

In 1919[3], no meeting being held in 1918, the Fendler-Stüber method was critically studied and a slight error was found, because of the action of the potassium permanganate on the caffeine during the process of purification when carried out at room temperature. Collaborative work was undertaken on a modification, providing for purification at the temperature of ice, the object of which was to eliminate this error. The results did not warrant the adoption of the modification as the caffeine obtained was inferior in purity to that obtained by the original treatment. The original method was retained as a tentative method with such other minor modifications as tended toward easier and more accurate manipulations. The maximum error found when the purification was conducted at room temperature was 1 per cent which, on a coffee containing 1.50 per cent of caffeine, (a maximum content in the experience of the referee) would mean an error of 0.015 per cent on the determination. This percentage of error is well within the limits of error of the method itself, as well as within the error of the personal equation, as shown by the various collaborative duplicate results reported in 1917 and 1919.

The advantages of the Fendler-Stüber method are: Rapidity, it being possible to make a determination in about 3 hours; ease of manipulation, no extensive apparatus being necessary; and the production of an exceptionally pure caffeine residue. Collaborative investigation has established its accuracy and general usefulness on green, roasted, and on the so-called "decaffeinated" coffees. In view of the marked advantages of the method and its accuracy, the slight error previously discussed not being considered of sufficient magnitude to condemn the method, it is believed that it should be retained, in the form adopted in 1919[4], as a tentative method.

At the last meeting of the association, the Power-Chesnut method[5] for caffeine was recommended to the referees on coffee and tea for study. These authors devised a practically new method although they termed it "An Improved Method for the Quantitative Determination of Caffeine in Vegetable Material". Each step of the method was studied by them and judged to be accurate. A series of control experiments, to ascertain whether caffeine was lost in any of the manipulations of the method,

[1] *J. Assoc Official Agr. Chemists*, 1917, 3: 21.
[2] *Z. Nahr. Genussm.*, 1914, 28: 9.
[3] *J. Assoc. Official Agr. Chemists*, 1921, 4: 526.
[4] *Ibid.*, 533.
[5] *J. Am. Chem. Soc.*, 1919, 41: 1298.

was carried out by the referee before collaborative samples were sent out.

The first step to receive attention was the alcoholic extraction of the sample. In order to determine whether the extraction had been complete, a delicate test for caffeine was desirable. Gomberg[1] found that Wagner's reagent used in the presence of hydrochloric acid would detect caffeine by precipitation of caffeine periodide in a dilution of 1 to 10,000. He also found that the reagent would not precipitate caffeine in the presence of fairly strong acetic acid. These findings were verified by the referee, using Wagner's reagent[2]. The delicacy 1 to 10,000 would allow the detection of 0.1 mg. in 1 cc. of solution. Two roasted and two unroasted coffees were extracted for 8 hours with alcohol as directed in the Power-Chesnut method. The residual coffees were then moistened with 10 per cent ammonium hydroxide and re-extracted with chloroform. After evaporation, the chloroform residues were dissolved in 1 cc. of water. To each solution Wagner's reagent and a few drops of dilute acetic acid were added. The slight precipitates formed were filtered off and the addition of 4 drops of hydrochloric acid failed to produce even a turbidity in any of the four filtrates. This indicates that the alcoholic extraction removed all the caffeine from the coffees as the test applied showed that chloroform failed to further extract caffeine or, at least, that the quantity so extracted was less than 0.1 mg.

The use of heavy magnesium oxide was shown by Power and Chesnut not to be detrimental to the complete recovery of caffeine and a reference[3] is cited by them wherein it is reported that magnesia has no action on caffeine at 100°C. Markownikoff[4] and Mulder[5] devised methods for the determination of caffeine wherein magnesia is employed. As none of these authors used the procedure of evaporation of an alcoholic extract in the presence of magnesia with subsequent solution of the caffeine in hot water, as provided in the method under consideration, this step was studied. Two samples of caffeine of 0.2000 gram each were dissolved in 50 cc. of alcohol and 50 cc. of water and 10 grams of heavy magnesium oxide were added. The resulting mixtures were evaporated to dryness on the steam bath, extracted with hot water, filtered, and washed with hot water until the filtrate measured 250 cc. On extraction of the filtrate with six successive 25 cc. portions of chloroform, evaporation of the chloroform, and drying, residues weighing 0.1998 and 0.1980 gram were obtained. This caffeine had an uncorrected melting point of 230°C. These recoveries indicate that the magnesia, as used in the method, is without action on the caffeine. The heavy

[1] *J. Am. Chem. Soc.*, 1896, 18: 331.
[2] *Assoc. Official Agr. Chemists, Methods.* 2nd ed., 1920, 292.
[3] *Bull. soc. chim.*, 1897, 3rd ser., 17: 597.
[4] *J. Russ. Phys.-Chem. Soc.* (*Phys. Pt.*), 1876, 5th ser., 8: 226.
[5] *Z. anal. Chem.*, 1873, 12: 107.

magnesium oxide used in these tests met the U. S. P. requirements with respect to soluble salts and carbonates.

The next step to be considered was the boiling of the filtrate from the magnesia treatment with 10 cc. of 10 per cent sulfuric acid for 30 minutes. The authors of the method found no action of sulfuric acid on caffeine. Zoller[1] and Costes[2] both propose methods for the determination of caffeine, using sulfuric acid in the process. Neither of them however, determined whether this acid is without action on the base. Therefore, the referee boiled 3 portions of caffeine of 0.2000 gram each in 200 cc. of water, increasing the quantity of acid in one case and lengthening the period of time in another case over that proposed in the method. The caffeine after treatment was extracted with six successive 25 cc. portions of chloroform, the chloroform evaporated and the residues weighed. The conditions of treatment and recoveries are given in the following table:

TABLE 1.

*Treatment of caffeine solution with sulfuric acid.*

| 10% SULFURIC ACID | TIME OF TREATMENT | WEIGHT OF CAFFEINE RESIDUE | UNCORRECTED MELTING POINT |
|---|---|---|---|
| *cc.* | *minutes* | *gram* | °*C.* |
| 10 | 30 | 0.1999 | 230 |
| 10 | 120 | 0.1998 | 230 |
| 40 | 30 | 0.1994 | 230 |

These results indicate that the treatment with sulfuric acid of the concentration and for the time specified in the method has no effect on the caffeine.

The final procedure specified in the method for purifying the caffeine is a treatment of the chloroform extract with dilute potassium hydroxide to remove coloring substances. Paul and Cownley[3] used dilute sodium hydroxide for the same purpose in a manner similar to that directed in the method under consideration. Dvorkowitsch[4] employed barium hydroxide in a method for the determination of caffeine but not in a similar manner. Neither of these authors did any work to determine whether treatment with alkali solution affected the caffeine. However, Power and Chesnut found no effect by the alkali when used as prescribed in this method. This finding was verified by the referee. Two samples of 0.2000 gram of caffeine were dissolved in 150 cc. of chloroform and the two solutions washed with 5 cc. of 10 per cent potassium

[1] *Neues Repertorium Pharm.*, 1871, 20: 457; *Ann.* 1871, 158: 180.
[2] *Ann. chim. anal.*, 1912, 17: 246.
[3] *Pharm. J.*, 1887, 3rd ser., 18: 417
[4] *Ber.*, 1891, 24: 1945.

hydroxide solution. The hydroxide was washed several times with small portions of chloroform which were added to the original chloroform solution. On evaporation and drying, residues of caffeine weighing 0.1990 and 0.1995 gram were obtained, having an uncorrected melting point of 230°C. It is evident that the alkaline washing is without effect on the caffeine.

The caffeine used in the preceding tests was Merck's U. S. P. which was recrystallized from water and dried at 100°C. Determination of nitrogen showed the caffeine to be 99.5 per cent pure. It had an uncorrected melting point of 230°C. Comparison of the melting points found on the caffeine residues obtained in the tests indicated that the caffeine was unaltered in the various procedures to which it was subjected.

Two samples of coffee, (A) a roasted Santos and (B) a coffee from which it was claimed that 95 per cent of the caffeine had been removed, were finely ground and sent out for collaborative tests. The following directions, which have been slightly changed, were sent to each collaborator. The changes are of a minor nature and in no way affect the important steps of the method.

### POWER-CHESNUT METHOD FOR THE DETERMINATION OF CAFFEINE IN COFFEE.

#### DETERMINATION.

Moisten 10 grams of the finely powdered sample with alcohol, transfer to a Soxhlet, or similar extraction apparatus, and extract with alcohol for 8 hours. (Care should be exercised to assure complete extraction.) Transfer the extract with the aid of hot water to a porcelain dish containing 10 grams of heavy magnesium oxide in suspension in 100 cc. of water. (This reagent should meet the U. S. P. requirements.) Evaporate slowly on the steam bath with frequent stirring to a dry, powdery mass. Rub the residue with a pestle into a paste with boiling water. Transfer with hot water to a smooth filter, cleaning the dish with a rubber-tipped glass rod. Collect the filtrate in a liter flask marked at 250 cc. and wash with boiling water until the filtrate reaches the mark. Add 10 cc. of 10% sulfuric acid and boil gently for 30 minutes with a funnel in the neck of the flask. Cool and filter through a moistened double paper into a separatory funnel and wash with small portions of 0.5% sulfuric acid. Extract with six successive 25 cc. portions of chloroform. Wash the combined chloroform extracts in a separatory funnel with 5 cc. of 1% potassium hydroxide solution. Filter the chloroform into an Erlenmeyer flask. Wash the potassium hydroxide with 2 portions of chloroform of 10 cc. each, adding them to the flask together with the chloroform washings of the filter paper. Evaporate or distil on the steam bath to a small volume (10–15 cc.), transfer with chloroform to a tared beaker, evaporate carefully, dry for 30 minutes in a water oven, and weigh. The purity of the residue can be tested by determining nitrogen and multiplying by the factor 3.464.

The results of the collaborators, appearing in Table 2, show that the method is well adapted for general analytical procedure, that good duplicates can be obtained and that the results of independent analysts agree as closely as could be expected.

TABLE 2.

*Determination of caffeine by the Power-Chesnut method.*

| COLLABORATOR | CAFFEINE BY WEIGHT | | | |
|---|---|---|---|---|
| | Sample A | | Sample B | |
| | Gravimetric | N × 3.464 | Gravimetric | N × 3.464 |
| | *per cent* | *per cent* | *per cent* | *per cent* |
| M. L. Offutt, Bureau of Chemistry, Washington, D. C. | 1.69 | 1.38 | 0.28 | 0.13 |
| | .... | .... | 0.32 | 0.15 |
| D. B. Scott, Bureau of Chemistry, Washington, D. C. | 1.55 | 1.48 | 0.24 | 0.17 |
| | 1.55 | 1.44 | 0.20 | 0.14 |
| J. I. Palmore, Bureau of Chemistry, Washington, D. C. | 1.50 | 1.40 | 0.22 | 0.15 |
| | 1.52 | 1.44 | 0.21 | 0.14 |
| R. E. Andrew, Agricultural Experiment Station, New Haven, Conn. | 1.51 | 1.47 | 0.21 | 0.17 |
| C. E. Shepard, Agricultural Experiment Station, New Haven, Conn. | 1.49 | 1.45 | 0.21 | 0.18 |
| Louis Pine, U. S. Food and Drug Inspection Station, New York, N. Y. | 1.49 | 1.41 | 0.20 | 0.18 |
| | 1.50 | 1.43 | 0.21 | 0.19 |
| C. W. Harrison, U. S. Food and Drug Inspection Station, Baltimore, Md. | 1.57 | .... | 0.22 | .... |
| H. J. Wichmann, U. S. Food and Drug Inspection Station, Denver, Colo. | 1.53 | .... | 0.21 | .... |
| J. H. Bornmann, U. S. Food and Drug Inspection Station, Chicago, Ill. | 1.39 | 1.32 | 0.21 | 0.17 |
| | 1.44 | 1.34 | 0.20 | 0.16 |
| H. A. Lepper. | 1.53 | 1.47* | 0.26 | 0.17* |
| | 1.50 | 1.42* | 0.25 | 0.17* |

*Determination made in the Nitrogen Laboratory of the Bureau of Chemistry, Washington, D. C

In Table 3, the results of the analysis of one sample of unroasted and one sample of roasted coffees used as collaborative samples in 1919, are given and comparison is made with the results obtained by the author by the Fendler-Stüber method.

TABLE 3.

*Determination of caffeine by the Power-Chesnut and Fendler-Stüber methods.*

| SAMPLE | POWER-CHESNUT METHOD | | FENDLER-STÜBER METHOD | |
|---|---|---|---|---|
| | Caffeine | | Caffeine | |
| | Gravimetric | N × 3.464 | Gravimetric | N × 3.464 |
| | *per cent* | *per cent* | *per cent* | *per cent* |
| Coffee, roasted | 1.21 | 1.10 | 1.19 | 1.16 |
| Coffee, unroasted | 1.28 | 1.20 | 1.34 | 1.26 |

The Power-Chesnut method is shown to be founded on accurate principles, to give results agreeing with those obtained by the tentative Fendler-Stüber method, to give good results by analysts, in general, and to work equally well on roasted, unroasted and on so-called "decaffeinated" coffees. It has the advantage of being a flexible method in that the amount of sample can be varied and it is adaptable to various

forms of vegetable material. This is the first year that the Power-Chesnut method has been studied by the association but it is felt that the results and conditions justify its adoption as an official method. The authors were members of this association when the method was devised after thorough investigation in the Phytochemical Laboratory of the Bureau of Chemistry. It has also been critically studied by the referee and collaborative results warrant its adoption. The method contains no radical departure from recognized principles and all the reagents employed have been used or suggested by two or more authors in connection with the determination of caffeine. This action is especially desirable as the determination of caffeine has received attention by the association for the past twelve years. The history of the determination of caffeine, both in and out of the association, shows that the large number of methods suggested are due in part to adoptions, revisions and modifications until nothing seems to remain to be done except revise and modify and it does not appear that further progress or advantage could be gained by continuing the study of the method. The action suggested in this report, if adopted, will provide the association with an official method of scientific accuracy and wide adaptability and with an accurate tentative method to be used when results are desired quickly, to be confirmed, if necessary, by the official method. If the association believes that the conditions do not warrant the adoption of the Power-Chesnut method as official, it is recommended that it be adopted as a tentative method and that further work on caffeine in coffee be discontinued until the methods for other constituents are improved.

## RECOMMENDATIONS.

It is recommended—

(1) That the Fendler-Stüber method for the determination of caffeine in coffee be retained as a tentative method and be designated as a method to be used when quick results are desired.

(2) That the Power-Chesnut method, page 271, be adopted as an official method.

(3) That the Stahlschmidt method for the determination of caffeine in coffee be dropped.

(4) That the referee on coffee next year study the methods for the determination of other constitutents.

# ROBUSTA COFFEE.

By ARNO VIEHOEVER and H. A. LEPPER (Bureau of Chemistry, Washington, D. C.).

## INTRODUCTION.

The designation "Robusta Coffee" is given to a product now grown in Java on a very large scale. It is, however, not identical with the coffee generally known as Java coffee, representing *Coffea arabica* L., and being the product, which, forty or more years ago, was the only coffee grown in Java. Since the eighties (1878) of the last century, different diseases, among them especially coffee blight, have more and more extinguished the plantations of *Coffea arabica*, and even those still existing in the mountainous districts diminish rapidly. Neither is Robusta coffee identical with Liberica coffee, which, although of somewhat inferior quality, proved more disease-resistant than *Coffea arabica* and was grown instead of the latter in considerable amounts until within recent years it was replaced by the new variety, *Coffea robusta*. This, as we shall see, is now grown in Java in larger amounts than both *Coffea arabica* and *Coffea liberica* Hier. together, due to its many good qualities—disease-resistancy, rapid growth, early and prolific yield, and its usefulness as a catch-crop in rubber, cocoanut and other plantations.

## ORIGIN, CLASSIFICATION, TERMINOLOGY.

Robusta coffee is of African origin and was found in the Eala district of the Belgian Congo by Ed. Luija, one of the travelers for L. Linden, Belgian horticulturist. Linden sold the plantlets grown from the seed in the years 1901 and 1902 to planters and the government in Java under the now well-established name *Coffea robusta* Linden.

The botanical classification is not fully settled. It is a disputed question whether *Coffea robusta* Linden, found native also in the Frencn Congo by M. Chevalier and sometimes referred to as *Coffea robusta* Chevalier, is a species different from *Coffea canephora*, as believed by Cramer[1], or a variety or form of *Coffea canephora* Pierre, indigenous to Central and West Africa, as pointed out by De Wildeman[2]. *Coffea laurentii*, found wild in the Congo region by Emil Laurent in 1918 according to De Wildeman, is nothing but a form of *Coffea canephora*, and thus closely related to *Coffea robusta*. Van Hall[3] states: "Perhaps two newly imported varieties named *Coffea laurentii* and *Coffea canephora* var. *sankuruensis* must be regarded as belonging to the robusta." According to Cramer[1] *Coffea laurentii*, *Coffea canephora* var. *sankuruensis*

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*Tea and Coffee Trade Journal*, 1918, 35: 418

[1] *Bull. assoc. planteurs Caoutchouc*, 1912, 4: 55.

[2] *Agri. Bull. Federated Malay States*, 1913, 1: 253.

and *Coffea canephora* var. *kwiluensis* or *kouilouensis*, probably belong to *Coffea robusta*. Wurth (See De Wildemann, 1912) considers that the group or type *robusta* represents the *canephora*, *quillou*, and *ugandae* varieties. Wester[1] similarly speaks of a *Coffea robusta* type or group, including the varieties *robusta*, *canephora*, *quillou* and *ugandae*. Cramer[2], while conceding that *Coffea robusta* is a mixture of different varieties, considers *Coffea canephora*, *Coffea ugandae*, and *Coffea quillou* as allied species rather than as varieties of *Coffea robusta*.

## PART I. BOTANICAL CHARACTERISTICS.

The Robusta group has not yet been subjected to a thorough study.

*Plants.*—The plants representing the *Coffea robusta* group are not of one uniform type, differing in size and shape of the leaves, fruits, etc. The following may be considered as general characteristics of the Robusta group according to Van Hall[3], Gallagher[4], and Cramer[2]:

The habit of the trees is much alike. They are early and strong bearers, the fruits are small and arranged in dense clusters, bearing often over sixty fruits. The leaves show rather more variety, but they are always larger than those of *Coffea arabica*, and more or less of the size of the *liberica* leaf, sometimes smaller, sometimes larger, but never of leathery appearance, and softer and weaker. The young leaves are green, not brownish, the basal part of the leafblade is emarginated toward the stalk. The flowers appear in thick clusters, and are large and broader petaled than those of *Coffea arabica*. The fruits, when unripe, are green and never orange colored; when almost ripe, vermillion-red; and when completely ripe, very dark red, with a bluish tinge. The berries are smaller and especially shorter than in true Java coffee. The pulpy substance of the berry shows very little development and, consequently, is difficult to remove, necessitating a longer fermentation than is needed with *arabica*.

The decided superiority of the *Coffea robusta* as a cropper over *Coffea arabica* is evident from the fact that in Java, under identical conditions, 53 to 97 grams of coffee beans per plant were obtained from *arabica* coffee, and 992 grams of *robusta*. A hybrid, *maragogipe*, obtained by grafting *Coffea arabica* on *Coffea robusta*, yielded 156 grams, thus demonstrating *Coffea robusta* to be a promising stock. According to Wester[4], Cramer found also that only 4 to 5 kilograms of fresh Robusta, but 5 to 6 kilograms of fresh Arabica fruits are required to make 1 kilogram of coffee.

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[1] *Philippine Agri. Rev.* 1916, 9: 121.
[2] *Tea and Coffee Trade Journal*, 1918, 35: 417.
[3] *Agri. Bull. Federated Malay States*, 1913, 1: 253.
[4] *Agri. Federated Malay States, Bull.* 7: (1910), 1.
[4] *Philippine Agri. Rev.*, 1915, 8: 45.

*Seeds.*—The Robusta beans require quick drying in order to loosen the silver skin. The fact that the silver skin is apparently difficult to remove is suggested from the appearance of commercial samples which contain a considerable number of beans showing remains of silver skin and resulting in a less uniform product. The sample of *Coffea robusta* obtained from the Netherlands East Indian Exhibit, San Francisco Exposition 1915, was practically free from silver skin.

The color, according to Hartwich and other writers, agrees in general with No. 297, the color tables of Klincksieck and Valette[1]. It is the characteristic coffee color, light to olive buff, with faint grayish, greenish or bluish tint.

The shape is that of the normal coffee bean, not many peaberries having been observed. The beans are said to be more convex on the curved side than those of *Coffea arabica*[2]. The writers have observed rather the reverse to be the case. The form, according to Cramer[3], is less oblong than in Java coffee; the circumference on the flat side of the bean is oval, not edged by straight lines. The average size of commercial samples examined is rather small, though varying considerably. Hartwich[4], states that he found a sample showing a length of 0.75 to 1.05 cm., a width of 0.6 to 0.7 cm., and a thickness of 0.35 to 0.5 cm.

The measurements made in the Pharmacognosy Laboratory of the Bureau of Chemistry by J. F. Clevenger and Ruth G. Capen are tabulated below. Ten seeds of each sample were examined.

TABLE 1.

*Latitude in size of Robusta coffee beans.*

| LENGTH | | | WIDTH | | | THICKNESS | | |
|---|---|---|---|---|---|---|---|---|
| Minimum | Average | Maximum | Minimum | Average | Maximum | Minimum | Average | Maximum |
| *cm.* | *cm.* | *cm.* | *cm.* | *cm.* | *cm* | *cm.* | *cm.* | *cm.* |
| 0.75 | 1.00 | 1.16 | 0.55 | 0.69 | 0.79 | 0.37 | 0.45 | 0.54 |
| 0.61 | 0.97 | 1.15 | 0.56 | 0.79 | 0.92 | 0.35 | 0.39 | 0.55 |
| 0.73 | 0.92 | 1.16 | 0.48 | 0.69 | 0.79 | 0.37 | 0.45 | 0.50 |
| 0.62 | 0.87 | 1.15 | 0.52 | 0.67 | 0.79 | 0.34 | 0.45 | 0.63 |
| 0.65 | 0.84 | 1.02 | 0.55 | 0.69 | 0.79 | 0.33 | 0.42 | 0.55 |
| 0.69 | 0.86 | 0.98 | 0.50 | 0.68 | 0.83 | 0.33 | 0.44 | 0.53 |
| 0.71 | 0.87 | 1.05 | 0.58 | 0.70 | 0.79 | 0.35 | 0.42 | 0.49 |
| 0.70 | 0.84 | 1.03 | 0.55 | 0.68 | 0.82 | 0.34 | 0.46 | 0.64 |
| | 0.90 | | | 0.70 | | | 0.43 | |

The latitude in sizes thus determined was a length of 0.61 to 1.16 cm., a width of 0.48 to 0.82 cm. and a thickness of 0.33 to 0.64 cm. These

[1] Paul Klincksieck and Th. Valette. Code des Couleurs. 1908, 56.

[2] *Tea and Coffee Trade Journal*, 1915, 29: 223.

[3] *Ibid.*, 1918, 35: 417.

[4] A. Beythien, C. Hartwich, and M. Klimmer. Handbuch der Nahrungsmittel-Untersuchung, 1913-1915, 2: 309.

sizes fall within the sizes observed in varieties belonging to *Coffea arabica* and *liberica*.

*Endosperm.*—The manner of folding of the endosperm observed upon a cross section of the bean is considered of value in the identification and differentiation of coffee. Hartwich points out that the endosperm of Robusta coffee shows a characteristic recurving, with hook, which occurs in most of the beans of Robusta. The writers have observed this recurving and can thus confirm Hartwich's statement. Inasmuch as the curving of the folded edge of the endosperm changes naturally with the place where the section is made, the authors advise making the section through the middle of the bean. Pending further investigation of other varieties of the group Robusta, it is believed that this characteristic can be used with advantage.

In contrast to the bean of *Coffea robusta* those of *Coffea arabica* show a double or a recurved edge of the endosperm but usually without the hook. *Coffea liberica* shows a simple curve, also without a hook. (See Illustration I, A R L.)

*Embryo.*—The size of the embryo, and the relative size of the cotyledon to hypocotyl is also considered of diagnostical value. Hartwich reports the size of the embryo of the Robusta coffee to be 0.6 cm. and the relation in size of cotyledon to hypocotyl 1 to 1.76. Three samples of Robusta coffee were examined in the Pharmacognosy Laboratory. For purposes of comparison three samples of *Coffea arabica* (Mocha Arabica) and two of *liberica* were also examined. The results are given in Table 2.

TABLE 2.

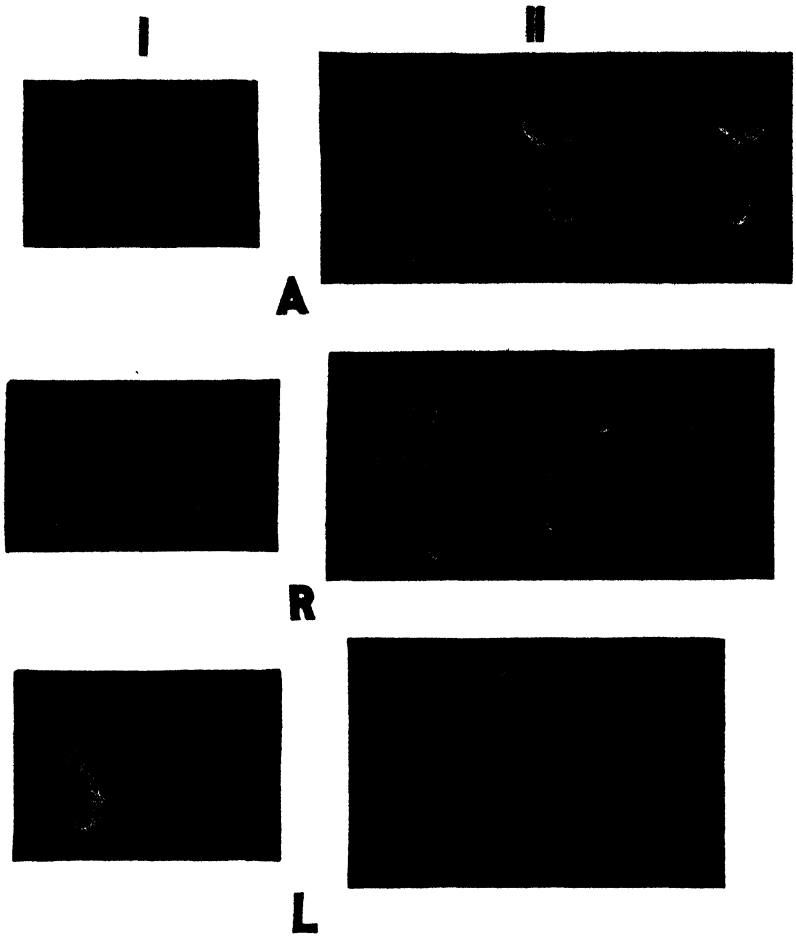
*Comparative sizes of hypocotyl and cotyledon*.*

| NAME OF SAMPLE | HYPOCOTYL | | | COTYLEDON | | | RELATION |
|---|---|---|---|---|---|---|---|
| | Minimum | Average | Maximum | Minimum | Average | Maximum | |
| | cm. | cm. | cm. | cm. | cm. | cm | cm. |
| Mocha Arabica | 0.17 | 0.27 | 0.31 | 0.11 | 0.17 | 0.21 | 1: 1.6 |
| Bourbon Santos | 0.25 | 0.27 | 0.31 | 0.14 | 0.16 | 0.20 | 1: 1.7 |
| Bourbon Santos | 0.20 | 0.23 | 0.28 | 0.13 | 0.14 | 0.19 | 1: 1.67 |
| Sumatra Robusta | 0.29 | 0.34 | 0.37 | 0.14 | 0.16 | 0.19 | 1: 2.08 |
| Java Robusta | 0.31 | 0.34 | 0.37 | 0.18 | 0.18 | 0.19 | 1: 1.8 |
| Sumatra Robusta | 0.27 | 0.32 | 0.37 | 0.15 | 0.17 | 0.22 | 1: 1.9 |
| Native Liberica | 0.39 | 0.45 | 0.57 | 0.14 | 0.16 | 0.21 | 1: 2.7 |
| Liberica, Venezuela | 0.49 | 0.51 | 0.55 | 0.17 | 0.19 | 0.22 | 1: 2.6 |

*Ten beans of each sample were used.

The results show that the embryos of *Coffea robusta* are distinctly smaller than those of *Coffea liberica*, and also, in average, a little larger

*Differentiating characteristics of the coffee beans.*




I. Cross section of bean. Approximately X3.
II. Embryo. Approximately X7.
A. *Coffea arabica.*
R. *Coffea robusta.*
L. *Coffea liberica.*

than those of *arabica.* (See Illustration II, A R L.) This fact is in accordance with previous findings and constitutes a means to distinguish Robusta coffee from other varieties.

*Stone cells.*—The stone cells represent sclerenchyma fibers or sclerenchyma cells present in the silver skin. Their length may reach, according to Hartwich, 700 microns. A few measurements have shown sclerenchyma fibers about 500 microns in the furrow of the bean and 750 microns on the convex side of the bean. A tabulation of data

given in literature, as well as those found by the authors, is given in Tables 3 and 4.

From these results, and especially from the writers' detailed findings, the following is evident:

The sizes of stone cells vary markedly in the furrow of the cotyledon from those of the convex side of the beans. The cells of *Coffea liberica* appear to differ in all particulars sufficiently from both *Coffea robusta* and *arabica* to enable ready differentiation. The sclerenchyma cells of *Coffea robusta* differ only comparatively slightly from *Coffea arabica*. The sizes are about the same, especially as far as maximum length is concerned. The cell wall is a little thinner in the case of *Coffea robusta* cells, and the ends of the cells are more pointed. Further work on other varieties of the group *robusta*, as well as of the species *arabica*, will have to be done before a final statement can be made as to the usefulness of the sclerenchyma cells of the seed coat for the purpose of differentiation.

## PART II. CHEMICAL CHARACTERISTICS.

Chemical analyses of seven samples of Robusta coffee were made, and the results are given in Table 5. The origin of these samples is also given in the table. For the sake of comparison, analyses of other varieties of coffee, some of which were taken from the literature, are included. Absolute ether was used for the determination of fatty material instead of petroleum ether, as this had previously been used by one of the authors on other varieties with which comparison is made. The Power-Chesnut method, page 271, for caffeine was used for the determination of this constituent.

The comparison with other varieties of coffee shows, in general, that the ether extractives are lower in Robusta than in other varieties, and that the caffeine is higher than that of South American varieties as represented by the kinds listed.

The sample of Robusta obtained from the San Francisco Exposition was subjected to the Power-Chesnut procedure for caffeine. The crude residue was dissolved in 5 cc. of 0.2 N sulfuric acid. A sufficient amount of Wagner's reagent was added to precipitate the caffeine periodide. The precipitate was dissolved in sodium thiosulfate solution and the solution extracted with chloroform. After evaporation of the chloroform a nearly white residue was obtained. This residue had a melting point of 228.5°C. (uncorrected).

The test described by Mulliken[1] for the specific characterization of caffeine, which gives the uncorrected melting point of caffeine as 229.3° to 30.3°C., was made. The derivative $C_8H_{10}O_2N_4HgCl_2$ was obtained, similarly to one from a sample of Merck's caffeine which had been recrystallized from water and dried. The mercury complex obtained

[1] S. P. Mulliken. A Method for the Identification of Pure Organic Compounds. 1st ed., 1916, 2: 215.

TAB

*Characteristics of sclerenchyma*

(General

| AUTHORS AND ANALYSTS | MAXIMUM LENGTH | MAXIMUM WIDTH | AVERAGE LENGTH | AVERAGE WIDTH |
|---|---|---|---|---|
| *Coffea arabica:* | *microns* | *microns* | *microns* | *microns* |
| C. Hartwich* | 700 | 51 | 520 | .... |
| A. E. Vogl† | 700 | 45 | 300 | 36 |
| A. L. Winton‡ | 1000+ | 50 | .... | .... |
| J. Moeller§ | 800 | 45 | .... | .... |
| C. Hartwich**†† | 730 | 65 | .... | 35 |
| R. G. Capen‡‡ | 700 | 43 | 400 | 35 |
| | 330 | 35 | 250 | 25 |
| *Coffea robusta:* | | | | |
| C. Hartwich* | 700 | .... | .... | .... |
| R. G. Capen‡‡ | 750 | 35 | 300 | 22 |
| | 500 | 32 | 200 | 25 |
| *Coffea liberica:* | | | | |
| C. Hartwich*‡‡ | 860 | 60.2 | 700 | 43 |
| | 960 | 60.2 | 700 | 51.6 |
| C. Hartwich§§ | 880 | 51.2 | 660–760 | .... |
| R. G. Capen‡‡ | 650 | 60 | 400 | 40 |
| | 310 | 75 | 300 | 56 |

*A. Beythien, C. Hartwich, and M. Klimmer. Handbuch der Nahrungsmittel-Untersuchung. 1913-1915, 2: 304, 307, 309.

†A. E. Vogl. Die Wichtigsten Vegetabilischen Nahrungs und Genussmittel. 1899. 299.

‡A. L. Winton. The Microscopy of Vegetable Foods 2nd ed., 1916, 432.

§J. Moeler. Mikroskopie der Nahrungs und Genussmittel. 1905, 406.

**C. Hartwich. Die Menschlichen Genussmittel. 1911, 278.

††Convex surface.

‡‡Convex surface for first set of determinations: furrow for second set.

§§C. Hartwich. Schweizerische-Wochenschrift für Chemie und Pharmacie. 1896, 475.

from the known caffeine had a corrected melting point of 250.8°C., while that obtained from the caffeine separated from the Robusta coffee was 250.2°C. A mixture of the two mercury salts gave a melting point of 250.5°C. Mulliken gives 251°C. as the melting point of the mercury complex. This confirms the assumption that the product obtained by the Power-Chesnut method and reported as caffeine was practically pure caffeine.

## COMMERCIAL DATA.

*Condition and quality.*—A sample of Robusta coffee grown in Java, obtained from the Netherlands Exhibit, San Francisco Exposition, 1915, represented coffee very fair in appearance. The beans were of a

LE 3.
*cells of silverskin (seed coat.)*
data.)

| MINIMUM LENGTH | MINIMUM WIDTH | MAXIMUM THICKNESS OF CELL WALLS | MINIMUM THICKNESS OF CELL WALLS | AVERAGE THICKNESS OF CELL WALLS | REMARKS |
|---|---|---|---|---|---|
| *microns* | *microns* | *microns* | *microns* | *microns* | |
| .... | .... | 15 | 4–6 | .... | |
| 75–90 | 15 | .... | .... | .... | |
| 100- | 15 | .... | .... | .... | Great variation in thickness of cell walls. |
| 70 | 15 | .... | .... | .... | |
| 90 | .... | .... | .... | 10–12 | Cells usually longer and thicker on convex surface. |
| 210 | 13.5 | 13.5 | 4 | 8 | |
| 175 | 13.5 | 10 | 4 | 8 | |
| .... | .... | .... | .... | .... | Cell walls slightly thinner and ends more pointed than Arabica. |
| 95 | 13.5 | 13.5 | 2.7 | 5.4 | |
| 80 | 13.5 | 10.8 | 2.7 | 5.4 | |
| .... | .... | .... | .... | .... | Walls thinner than Arabica. |
| .... | .... | .... | .... | .... | |
| .... | .... | .... | .... | .... | |
| 250 | 30 | 18.5 | 8 | 13.5 | |
| 210 | 35 | 19 | 8 | 13.5 | |

high grade, evidently carefully cleaned and uniform. The size, as pointed out before, was larger than other samples of Robusta. The color of the seeds was light yellowish brown, and not greatly different from a sample of genuine Java coffee which came to the writers' attention at a previous time.

Samples collected by inspectors in different States of the United States were generally of fair quality. The seeds on the average, however, were rather small and not so uniform in appearance as might have been desired. With the exception of one or two, the samples contained only few imperfections and consequently graded above No. 8, representing the lowest grade of coffee accepted by the New York Coffee Exchange. The samples representing the two exceptions were evidently very poorly cleaned and, judging from a casual examination, would possibly not have passed Grade 8.

*Economic significance.*—Robusta coffee, at first, was not greatly valued, inasmuch as the beans were small and irregular and gave the market product an inferior appearance. Gradually, however, its good

TABLE

*Characteristics of sclerenchyma cells of*

*(Detailed*

(Analyst, R.

| *Coffea arabica** | | | | |
|---|---|---|---|---|
| VARIETY | LENGTH OF CELL | WIDTH OF CELL | THICKNESS OF CELL WALL | VARIETY |
| | *microns* | *microns* | *microns* | |
| Mocha (convex surface) | 700 | 30 | 4–5.4 | Java |
| Mocha (convex surface) | 510 | 35 | 8–16 | Java |
| Mocha (convex surface) | 360 | 27 | 10.8–13.5 | Java |
| Mocha (convex surface) | 400 | 32 | 8 | Java |
| Mocha (convex surface) | 270 | 35 | 5.4 | Java |
| Mocha (convex surface) | 210 | 43 | 8 | Java |
| Mocha (convex surface) | 273 | 40 | 8 | Java |
| Mocha (convex surface) | 350 | 30 | 8–13.5 | Java |
| Mocha (convex surface) | 675 | 32 | 5.4 | Java |
| Mocha (convex surface) | 450 | 27 | 5.4 | Java |
| Mocha (furrow) | 200 | 13.5 | 4.5–6.7 | Sumatra, (convex) |
| Mocha (furrow) | 260 | 24 | 5.4–8 | Sumatra, (convex) |
| | | | | Sumatra, (convex) |
| | | | | Sumatra, (convex) |
| Java (furrow) | 330 | 27 | 5.4–10 | Sumatra, (convex) |
| Java (furrow) | 175 | 35 | 5.4–10 | Sumatra, (convex) |
| | | | | Sumatra, (convex) |
| | | | | Sumatra, (convex) |
| Bourbon Santos | 320 | 13.5 | 4.5–6.7 | Sumatra, (convex) |
| Bourbon Santos | 300 | 24 | 5.4–8 | |
| | | | | Sumatra (furrow) |
| | | | | Sumatra (furrow) |
| | | | | Sumatra (furrow) |
| | | | | Sumatra (furrow) |
| | | | | Sumatra (furrow) |
| | | | | Sumatra (furrow) |
| | | | | Sumatra (furrow) |
| | | | | Sumatra (furrow) |
| | | | | Sumatra (furrow) |
| | | | | Unnamed sample |
| | | | | " (convex surface) |
| | | | | " (furrow) |
| | | | | Unnamed sample |
| | | | | " (convex surface) |
| | | | | " (furrow) |
| | | | | Unnamed sample |
| | | | | " (convex surface) |
| | | | | " (convex surface) |
| | | | | " (convex surface) |

*Stone cells located in direction to longer axis. Lumen and cell walls not easily differentiated. Pitts long and stretched. Ends pointed. Cells more irregular in shape in furrow.

†Stone cells occur usually in groups of 2, lying in one axis. Lumen and cell walls easily differentiated. Pitts round or oval. Cells more irregular in shape in furrow.

‡Groups of stone cells quite irregularly mixed. Lumen and cell walls easily differentiated. Pitts round or oval. Cell walls much thicker where pitts are numerous.

4.
*silver skin (seed coat.)*
*observations.)*
G. Capen.)

| *Coffea robusta*† | | | *Coffea liberica*‡ | | | |
|---|---|---|---|---|---|---|
| LENGTH OF CELL | WIDTH OF CELL | THICKNESS OF CELL WALL | VARIETY | LENGTH OF CELL | WIDTH OF CELL | THICKNESS OF CELL WALL |
| *microns* | *microns* | *microns* | | *microns* | *microns* | *microns* |
| 410 | 33 | 13.5 | Native (convex surface) | 600 | 35 | 11 |
| 130 | 21 | 6–8 | Native (convex surface) | 530 | 35 | 11 |
| 200 | 35 | 5.4 | Native (convex surface) | 250 | 38 | 11 |
| 200 | 24 | 5.4 | Native (convex surface) | 300 | 46 | 13.5 |
| 115 | 26 | 5.4 | Native (convex surface) | 300 | 40 | 8–13.5 |
| 190 | 30 | 4 | Native (convex surface) | 530 | 49 | 13.5 |
| 260 | 16 | 5.4 | Native (convex surface) | 540 | 60 | 13.5 |
| 300 | 27 | 5.4–13.5 | Native (convex surface) | 650 | 46 | 8 |
| 350 | 32 | 5.4–16 | | | | |
| 300 | 22 | 5.4 | | | | |
| | | | Native (furrow) | 235 | 54 | 13.5–16 |
| | | | Native (furrow) | 300 | 35 | 8–13.5 |
| 270 | 24 | 8 | Native (furrow) | 250 | 67 | 16–19 |
| 200 | 22 | 5.4 | Native (furrow) | 290 | 60 | 13.5–19 |
| 190 | 22 | 8 | Native (furrow) | 200 | 50 | 13.5 |
| 750 | 32 | 8 | Native (furrow) | 310 | 56 | 13.5 |
| 160 | 19 | 2.7–8 | Native (furrow) | 285 | 56 | 13.5 |
| 220 | 19 | 2.7–8 | Native (furrow) | 250 | 57 | 13.5–16 |
| 220 | 22 | 2.7–8 | Native (furrow) | 210 | 54 | 13.5 |
| 135 | 19 | 2.7–8 | Native (furrow) | 200 | 75 | 16 |
| 95 | 19 | 2.7–8 | | | | |
| | | | Venezuela | 390 | 30 | 13.5 |
| 500 | 13.2–29 | 4 | Venezuela | 310 | 46 | 13.5 |
| 80 | 13.2 | 5.4 | Venezuela | 430 | 43 | 13.5 |
| 100 | 21 | 5.4 | Venezuela | 300 | 32 | 13.5 |
| 130 | 21 | 5.4 | Venezuela | 270 | 43 | 13.5 |
| 350 | 30 | 10.8 | Venezuela | 420 | 40 | 13.5 |
| 160 | 16 | 5.4 | Venezuela | 430 | 40 | 13.5 |
| 160 | 32 | 5.4–8.1 | | | | |
| 220 | 21 | 8.1 | | | | |
| 85 | 13–27 | 5.4 | Java | 180 | 46 | 13.5 |
| | | | Java | 135 | 48 | 13.5 |
| | | | Java | 150 | 54 | 13.5 |
| 375 | 19 | 8 | Java | 95 | 46 | 13.5 |
| 300 | 19–24 | 8 | | | | |
| 375 | 19 | 5.4–8 | | | | |
| 300 | 19 | 5.4–8 | | | | |
| 450 | 27 | 10–16 | | | | |
| 480 | 27 | 8 | | | | |
| 420 | 19 | 5.4–8 | | | | |

TABLE 5.

*Comparison of the analysis of Robusta coffee with other varieties of coffee.*

(Results expressed as per cent by weight.)

| KIND | MOISTURE OVEN AT 105° C.* | ABSOLUTE ETHER EXTRACT | COLD WATER EXTRACT* | CAFFEINE N×3.464 | MOISTURE-FREE BASIS | | |
|---|---|---|---|---|---|---|---|
| | | | | | Ether extract | Water extract | Caffeine |
| Robusta | | | | | | | |
| Raw† | 8.15 | 9.79 | 27.92 | 1.13 | 10.66 | 30.40 | 1.23 |
| Raw‡ | 8.09 | 8.65 | 27.20 | 1.79 | 9.41 | 29.60 | 1.95 |
| Roasted | 6.14 | 7.09 | 21.03 | 2.04 | 7.55 | 22.40 | 2.17 |
| Robusta | | | | | | | |
| Raw‡ | 7.02 | 9.14 | 28.56 | 1.95 | 9.83 | 30.72 | 2.10 |
| Roasted | 5.65 | 10.77 | 23.54 | 1.92 | 11.41 | 24.95 | 2.03 |
| Robusta | | | | | | | |
| Raw‡ | 7.65 | 7.53 | 27.79 | 1.66 | 8.15 | 30.09 | 1.80 |
| Roasted | 5.55 | 9.64 | 22.41 | 1.97 | 10.21 | 23.73 | 2.09 |
| Java roasted§ | 3.38** | 12.81†† | 23.08 | 1.30 | 13.26 | 23.89 | 1.35 |
| Java Arabica | | | | | | | |
| Raw‡‡ | 11.24 | 13.63 | 33.52 | 1.16 | 15.36 | 37.76 | 1.31 |
| Roasted | 5.64 | 14.20 | 27.40 | 1.57 | 15.05 | 29.04 | 1.66 |
| Java Liberica | | | | | | | |
| Raw‡‡ | 11.40 | 12.19 | 35.16 | 1.59 | 13.76 | 39.68 | 1.79 |
| Roasted | 3.98 | 13.13 | 34.17 | 2.19 | 13.67 | 35.58 | 2.28 |
| Mocha roasted§ | 3.47** | 14.83†† | 22.82 | 1.15 | 15.36 | 23.64 | 1.19 |
| Porto Rico roasted§ | 1.31** | 13.49†† | 22.89 | 1.30 | 13.92 | 23.62 | 1.34 |
| Rio No. 4 | | | | | | | |
| Raw§§ | 5.92 | 14.54 | 30.49 | 1.08 | 15.45 | 32.41 | 1.15 |
| Roasted | 3.05 | 14.43 | 29.98 | 1.24 | 14.88 | 30.92 | 1.28 |
| Rio No. 7 | | | | | | | |
| Raw§§ | 5.85 | 12.85 | 21.62 | 1.03 | 13.65 | 22.96 | 1.09 |
| Roasted | 3.94 | 14.05 | 22.06 | 1.31 | 14.63 | 22.96 | 1.36 |
| Santos roasted§ | 1.53** | 14.09†† | 21.74 | 1.18 | 14.67 | 22.63 | 1.23 |
| Victoria | | | | | | | |
| Raw§§ | 5.88 | 14.19 | 27.45 | 0.87 | 15.08 | 29.16 | 0.92 |
| Roasted | 2.88 | 15.37 | 22.06 | 1.09 | 15.83 | 22.71 | 1.12 |

*Assoc. Official Agr. Chemists, Methods 2nd ed., 1920, 269.

†From Netherland's East Indian Exhibit, San Francisco Exposition, 1915.

‡Collected by food and drug inspectors from coffee roasters.

§Average of 3 samples analyzed by H. C. Lythgoe. U. S. Bur. Chem. Bull. 90: (1905), 43.

**Water oven 3 hours at 100° C.

††Petroleum ether extract.

‡‡Analyzed by W. L. A. Warnier, *Pharm. Weekblad*, 1899, 36th year, No. 13; *Z. Nahr. Genussm.*, 1900, 3: 255.

§§Analyzed by H. A. Lepper. Reported by H. M. Loomis, *J. Assoc. Official Agr. Chemists*, 1920, 3: 502.

qualities were recognized; namely, its early bearing, prolific yields, resistance to the coffee blight, leaf disease (*Hemileia vastatrix*), and its comparative independence of climatic and soil conditions.

*Yield per acre.*—Cramer[1] reports that a crop of over 1520 pounds per acre may be expected under favorable conditions for *Coffea robusta*, and more than 4000 pounds for *Coffea quillou*, which is often included in the Robusta group.

[1] *Tea and Coffee Trade Journal*, 1918, 35: 417, 420.

*Extent of cultivation.*—The extent to which Robusta coffee has, within recent years, been grown in Java, may be seen from Table 6, which also gives data as to yields of *Coffea arabica* and *liberica:*

TABLE 6.
*Production of coffee in Java*.*

| YEAR | ARABICA | LIBERICA | ROBUSTA |
|---|---|---|---|
| | *kilos* | *kilos* | *kilos* |
| 1910 | 4,552,000 | 4,146,000 | 1,861,000 |
| 1911 | 6,177,000 | 3,661,000 | 7,666,000 |
| 1912 | 11,631,000 | 3,339,000 | 15,557,000 |
| 1913 | 4,555,000 | 3,123,000 | 18,207,000 |
| 1914 | 11,941,000 | 2,227,000 | 34,268,000 |

**Philippine Agri. Rev.*, 1916, 9: 120.

Further interesting data concerning the production of the different varieties for the years 1918, 1919, and 1920, showing the greatly predominating growth of *Coffea robusta* are tabulated in an article by Fowler[1]. In the same article the author states the following with regard to the acreage used for the cultivation of coffee:

According to statistics issued in September, 1919, by the Dutch East Indies Government, there were 144,663 hectares (357,469 acres) planted to coffee in the Dutch East Indies. Of this area 120,910 hectares (298,774 acres), representing 83½ per cent of the total, were in Robusta; 8,005 hectares (19,780 acres), or 5½ per cent, in Java (Arabica); 6,567 hectares (16,228 acres) or 4½ per cent, in Liberica; and the remainder, 9,181 hectares (22,687 acres) in various minor varieties.

*Grading.*—According to Trade Commissioner Fowler[2], fermented beans in Robusta, called "stinkers" by the trade, have given Robusta coffee a bad name in the American market. A first requisite is that this grade shall be entirely free from these defective beans. One-half per cent of broken and black beans are allowed in the grades exported to the United States. "Export quality" which is the only grade exported is "double picked".

*Extent of importation.*—From an importer of Robusta coffee it was learned that during the year 1919 approximately one million bags (about 136,000,000 pounds) of Robusta coffee were imported into the United States and it was prophesied that the amount will be still larger in 1920. The coffee is used, as far as can be learned, especially in the Western and Middle Western States.

*Utilization.*—Robusta coffee is used as such alone, or is blended with South American or other varieties of coffee. Some men in the coffee

[1] *Tea and Coffee Trade Journal*, 1920, 39: 300.
[2] Commerce Reports, November 11, 1920, 682.

trade consider the use of Robusta coffee as a filler if blended with other varieties; this opinion, again, is not general.

*Price.*—With regard to the price, the following quotations which indicate that the coffee, while not a very high-priced product, has a distinct and appreciable market value, have been taken from the literature and from trade statements: "$25 per 100 kilograms[1]". "Washed Robusta Coffee, quoted at 19¼ cents, and Santos at 24½ cents[2]".

*Flavor and taste.*—Of interest is the discussion in literature, as well as the trade opinion, concerning the flavor and taste of Robusta coffee. Before quoting a few detailed statements, it may be said that there is considerable difference of opinion; some consider the flavor and taste very desirable, others consider both quite inferior. It is evident that the degree of roasting and the preparation of the coffee as a beverage have considerable influence, and in order to bring out the cup quality of Robusta coffee it may be necessary to modify the preparation of the coffee for drinking, in order to get the best results. In Java, a concentrated cold water extract of the Robusta coffee is made by means of percolators. This extract is served with hot milk.

*Trade opinion in America.*—Of further interest is a statement published in 1912, concerning a decision of the New York Coffee Exchange[3]:

> In February 1912 an important decision was taken by the Board of Managers of the Coffee Exchange of the City of New York who decided in a special meeting to boycott the *robusta* from the American market.
>
> This measure seems to have been a consequence of the anxiety of coffee merchants in view of the possibility that *robusta* coffee, if tendered for delivery on a contract on the exchange, might have to be accepted in such a contract, while it is considered an undesirable property owing to its status as a poor seller. (9th Annual Report of the Nederland Chamber of Commerce in America.)
>
> Certainly the unfavorable opinion about *robusta*, which the American coffee merchants volunteer in their reports, is quite different from the opinion of the European experts, and it is very probable that they only regard the *robusta* as a dangerous competitor of the Santos coffee, over which the American merchants have a control, while the *robusta* imports are not under their control.

Ukers[4] and Salak[5] pointed out that the New York Coffee Exchange considered Robusta coffee as "a practically worthless bean".

*Opinion of coffee dealers.*—The following statements, made by representatives of the American coffee trade, taken at random, illustrate the present contradictory attitude:

---

[1] *Philippine Agri. Rev.*, 1916, **9**: 122.
[2] A. L. Sullivan. (Private communication.)
[3] *Agri. Bull. Federated Malay States*, 1913, **1**: 257.
[4] *Tea and Coffee Trade Journal*, 1912, **22**: 227.
[5] *Ibid.*, 1915, **29**: 223.

It is a neutral coffee which can be used in a blend with other coffees, since it will not affect the flavor.

Has not the value we desire in our coffee.

It is tasteless and bodyless.

Should be taken out and dumped in the Chesapeake Bay.

Really better than low grade Santos or Rio. If properly selected it is a very fair coffee, but like some others, there are all grades, some not worth anything.

Fancy in style and cup quality.

Robusta has been tested out and has wonderful cup value.

Robusta has no objectionable features like Rio and Santos.

Low grade Robusta (unwashed) was found superior to low grade Santos.

Robusta coffee has real value of its own, and should stand on this.

It is a good coffee, sweet and mild, better than Rio. It is superior to Nos. 6 and 7 of inferior varieties.

I am a firm believer in Robusta coffee. It has great cup value and is better than Rio, Victoria, and Central American coffees.

*Quotations from literature.*—The following quotations are included, since they give the opinion, mainly, of men who have given extensive attention to Robusta coffee in regions where it grows:

While in the drying house the coffee must be often moved so as to get a regular drying. Coffee so prepared and dried keeps its bluish color long and has a good flavor.

Must be heated and ground in a manner somewhat different from other coffees. Experts are inclined to put it nearly on a level as to quality with best Santos[1].

Its one defect is that it is not equal in quality to the better grades of the other types and therefore commands a lower price than the Arabian and Liberica[2].

The appearance of the average marketable Robusta is not very beautiful; the beans are small and irregular, and the average product shows little uniformity.

There are, however, great differences between the many different types of Robusta. Some of them have comparatively large beans, larger even than Arabica, others again have very small ones.

As regards the quality, though being inferior to Java-Arabica, the taste is generally considered to be good and superior to the ordinary Arabica sorts, as Santos.

It is objected that the berries of the Robusta group and of other African coffees are small in size and inferior in flavor; but the continually increasing quantities of these coffees sold in Holland, and the satisfactory prices they fetch show that the public is beginning to appreciate them. No objections will be made to the size of the berries when by means of careful cultivation and especially of right preparation, a coffee is obtained equal in flavor to the (old) Java and Arabian coffee[3].

According to Salak[4] the quality of well prepared Robusta coffee is approximately that of middling Arabian coffee.

The writers have carried out a number of cup tests, made as is customary in the coffee trade, of various samples of Robusta coffee. Even though the beverages thus obtained were not made according to the method followed in the Dutch East Indies, the results as to the quality of taste and flavor of Robusta coffee were distinctly favorable.

---

[1] *Dept. Agri. Federated Malay States*, Bull. 7: (1910), 5.

[2] *Philippine Agri. Rev.*, 1916, 9: 123.

[3] *Ibid.*, 1915, 8: 46.

[4] *Tea and Coffee Trade Journal*, 1915, 29: 223.

SUMMARY.

The time when coffee could be limited to beans obtained from plants of *Coffea arabica* and *Coffea liberica* has passed. Other species, with qualities which make them desirable, even in preference to the well reputed named ones, have been discovered and cultivated. Among them, the species or group of *Coffea robusta*, has attained a great economic significance and is grown in increasing amounts. While it has, as reports seem to indicate, not as yet been possible to obtain a strain that would be as desirable in flavor as the old "standard" *Coffea arabica*, well known as Java or "Fancy Java" coffee, its merits have been established.

The botanical origin is not quite cleared up and the classification of the varieties belonging to the Robusta group deserves further study. Anatomical means of differentiating Robusta coffee from other species or groups may be applied as distinctly helpful. It appears that the length of the embryo and the relation in size of cotyledon to hypocotyl, the folding of the endosperm, if recurved and showing a hook, and to some extent also the structure of the stone cells can be used as diagnostic characteristics in the identification of Robusta coffee. The seeds, on an average, are rather small, but may also attain fair sizes.

As is usual in most of the coffee species, caffeine is present. The amount appears to be, on an average, somewhat larger (even exceeding 2.0 per cent) than in other South American coffee species. In no instance, however, did the amount exceed the maximum limits observed in coffee in general.

The ether extractives were lower in *Coffea robusta* than in the other varieties named in Table 4. Further data on Robusta coffee relative to moisture, cold water extract, etc., were also determined, but did not show any marked difference from the other coffees examined.

Due to its rapid growth, early and prolific yield, resistance to coffee blight, and many other desirable qualities, *Coffea robusta* has established "its own". In the writers' judgment, Robusta coffee deserves consideration and recognition.

## REPORT ON TEA.

By E. M. Bailey (Agricultural Experiment Station, New Haven, Conn.), *Referee.*

A study of methods for the determination of caffeine in tea led to the following recommendation last year[1]:

That the modified Stahlschmidt method, as it now appears tentatively, with the exception that the caffeine residue be dried at 100°C. instead of 75°C., be made official for the determination of caffeine in tea.

[1] *J. Assoc. Official Agr. Chemists*, 1921, 4: 538.

Attention was also called to the work of Power and Chesnut[1] and it was voted that the referee on tea consider this method in his work this year. This suggestion has been carried out and the method, without material change, has been found to be very satisfactory, yielding caffeine of a high degree of purity. In addition, the Stahlschmidt method has been improved so that caffeine of a higher degree of purity than before is obtained. A new method has been evolved, which combines the most desirable points of the Power-Chesnut and the Stahlschmidt methods, which is shorter than either of those methods and which gives equally satisfactory results.

## COLLABORATION.

The work this year has been done with the collaboration of R. E. Andrew and C. E. Shepard, Agricultural Experiment Station, New Haven, Conn., and H. A. Lepper, Bureau of Chemistry, Washington, D. C.

## FURTHER STUDY OF METHODS FOR THE DETERMINATION OF CAFFEINE.

### *Improved Stahlschmidt Method.*

Since it was noted in the referee's report last year that caffeine obtained by the Fendler-Stüber method was of a slightly greater degree of purity than that yielded by the Stahlschmidt method it was thought that the latter method might be improved by introducing some modification to further purify the caffeine residue. The use of a dilute solution of potassium permanganate for this purpose in the case of coffee has been shown to result in a slight loss or destruction of caffeine[2]. Therefore, it was suggested by the writer and Shepard that dilute potassium hydroxide might be used for the purpose. This procedure is used by Power and Chesnut who have shown that no loss of caffeine results from the treatment and the writer has further tested this point as follows:

A small quantity (0.2 gram) of caffeine (99.4 per cent pure) was dissolved in 100 cc. of chloroform and the solution shaken in a separatory funnel with 5 cc. of 1% potassium hydroxide. After allowing the liquids to separate the chloroform was drawn off, the aqueous solution in the separatory funnel washed with chloroform in two portions of 10 cc. each, and these washings added to the main extract. Most of the chloroform was then removed by distillation, the residual portion transferred to a small tared flask, evaporated, dried at 100°C. and weighed.

Caffeine taken, 0.2000 gram; found, A, 0.1991; B, 0.1999.

By treating the combined chloroform extracts, as obtained in the modified Stahlschmidt method, with 5 cc. of 1 per cent potassium

[1] *J. Am. Chem. Soc.*, 1919, 41: 1300.
[2] *J. Assoc. Official Agr. Chemists*, 1921, 4: 526.

hydroxide, caffeine residues of a high degree of purity are obtained as shown by Table 1.

TABLE 1.

*Purification of caffeine by treatment with potassium hydroxide.*

| DESCRIPTION OF SAMPLE | WITHOUT POTASSIUM HYDROXIDE TREATMENT | | WITH POTASSIUM HYDROXIDE TREATMENT | |
|---|---|---|---|---|
| | By weight | From nitrogen | By weight | From nitrogen |
| | *per cent* | *per cent* | *per cent* | *per cent* |
| Black tea (4) | 2.96 | 2.77 | 2.83 | 2.81 |
| | . . . | . . . . | 2.89 | 2.87 |
| Green tea (5) | 1.86 | 1.73 | 1.65 | 1.59 |

The caffeine residues so obtained were practically free from color and their purity is further indicated by the close agreement between results for caffeine by weight and from nitrogen.

*Power-Chesnut Method*[1].

Extract 10 grams of material for 8 hours in a Soxhlet apparatus with hot 95% alcohol. Add the alcoholic extract to a suspension of 10 grams of heavy magnesium oxide in 100 cc. of water, rinse the flask with a little hot water, and add the rinsings to the mixture. Allow the mixture to evaporate slowly on a boiling water bath, with frequent stirring, until the alcohol is removed and a nearly dry powdery mass is obtained. Transfer the mass to a smooth filter by means of a sufficient amount of hot water, cleaning the container thoroughly. Wash the material on the filter with successive portions of hot water until the filtrate measures 250 cc. Add 10 cc. of 10% sulfuric acid to the filtrate contained in a flask of suitable size, place a funnel in the neck of the flask, boil cautiously until danger of frothing is passed and continue active boiling for 30 minutes. Allow the solution to cool and filter into a separatory funnel through a double moistened filter and wash the flask and filter with small portions of 0.5% sulfuric acid. Shake the clear acid filtrate with 6 successive 25 cc. portions of chloroform, collecting the several extracts in a second separatory funnel. Treat the combined chloroform extracts with 5 cc. of a 1% solution of potassium hydroxide and allow the chloroform to completely subside. Draw off the chloroform into a suitable flask, filtering through a dry paper or pledget of cotton inserted in the stem of the separatory flask. Wash the alkaline liquid remaining in the separatory funnel with two portions of chloroform, also washing the filter if used, and unite the washings with the main bulk of chloroform solution. Distil the solvent to a small volume, transfer to a tared beaker, evaporate to dryness, further dry for 1 hour at 100°C., cool and weigh.

To test the purity of the residue, determine nitrogen therein and calculate caffeine by the factor 3.464.

The authors have shown: (1) That extraction of caffeine from the material under examination is complete; (2) that no loss of caffeine results from the treatment with magnesia, provided the same is free

[1] *J. Am. Chem. Soc.*, 1919, **41**: 1300.

from appreciable amounts of sodium carbonate; (3) that no loss results from boiling the aqueous solution of caffeine with dilute sulfuric acid; and (4), that no loss results from the treatment of the chloroform solution of caffeine with dilute potassium hydroxide. The writer has corroborated this last point by an experiment previously cited, page 289.

Results by this procedure, compared with those by the Stahlschmidt method as modified this year, are shown in Table 2.

TABLE 2.

*Comparison of the Stahlschmidt and the Power-Chesnut methods for caffeine in tea.*

| DESCRIPTION OF SAMPLE | STAHLSCHMIDT METHOD | | POWER-CHESNUT METHOD | |
|---|---|---|---|---|
| | By weight | From nitrogen | By weight | From nitrogen |
| | *per cent* | *per cent* | *per cent* | *per cent* |
| Black tea (4) | 2.83 | 2.81 | 3.06 | 2.99 |
| | 2.89 | 2.87 | 3.05 | 3.03 |
| | 2.86* | 2.84* | 3.05 | 2.95 |
| Green tea (5) | 1.64 | 1.63 | 1.61 | 1.55 |
| | 1.65 | 1.59 | 1.69 | 1.60 |

*Acid hydrolysis modification.

The figures by the two methods are in substantial agreement and the caffeine residues are of about an equal degree of purity. In the Power-Chesnut method the aqueous solution of caffeine is treated with dilute sulfuric acid to hydrolyze possible saponin complexes and it was thought that the slightly higher results in the case of Sample 4 might be due to this feature of the method. Accordingly, the Stahlschmidt method was repeated, introducing this acid hydrolysis, but the results showed no increase in caffeine yield. However, the authors regard this step as important and it is no doubt a wise provision, although the necessity for it may not be apparent in every instance. The method possesses an advantage in that the use of lead and its subsequent removal is avoided; but your referee is still of the opinion that, so far as tea is concerned, boiling water is a better initial solvent since it extracts caffeine completely and materially simplifies the subsequent procedure.

*Proposed New Method.*

It therefore occurred to the writer and R. E. Andrew to extract the caffeine directly by boiling with water, in the presence of magnesia, make up to volume, take an aliquot portion and proceed as in the Power-Chesnut method, thus combining the best features of that method and of the Stahlschmidt method.

A procedure was finally evolved as follows:

To 5 grams of material in a 500 cc. graduated flask add 10 grams of heavy magnesium oxide and 200 cc. of distilled water. Boil gently over a low flame for 2 hours, using a small bore glass tube 30 inches long as a condenser. Cool, dilute to volume and filter through a dry paper. Take an aliquot portion of 300 cc., equivalent to 3 grams of original material, in an Erlenmeyer flask of 1 liter capacity, add 10 cc. of a 10% solution of sulfuric acid and boil until the volume is reduced to about 100 cc. Filter into a separatory funnel, washing the flask with small portions of 1% sulfuric acid, and shake six times with chloroform using 25, 20, 15, 10, 10, 10, cc. portions. Treat the combined extracts with 5 cc. of a 1% solution of potassium hydroxide, when the liquids have completely separated, draw off the chloroform layer into a suitable flask or beaker. Wash the alkaline solution in the separatory funnel with two portions of chloroform of 10 cc. each and unite the washings with the main bulk of extract. Evaporate or distil off the chloroform to a small bulk, transfer to a tared flask, evaporate to dryness, and further dry in a water oven at 100°C. to constant weight.

If desired, transfer the residue thus obtained to a digestion flask with successive small portions of sulfuric acid and determine nitrogen by the Kjeldahl method, calculating caffeine from nitrogen by the factor 3.464.

The results as compared with the improved Stahlschmidt method and with the Power-Chesnut method are shown in Table 3.

TABLE 3.

*Comparison of the Stahlschmidt, Power-Chesnut, and proposed methods for caffeine in tea.*

| DESCRIPTION OF SAMPLE | STAHLSCHMIDT METHOD | | POWER-CHESNUT METHOD | | PROPOSED METHOD | |
|---|---|---|---|---|---|---|
| | By weight | From nitrogen | By weight | From nitrogen | By weight | From nitrogen |
| | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* |
| Black tea (4) | 2.83 | 2.81 | 3.06 | 2.99 | 2.98 | 2.86 |
| | 2.89 | 2.87 | 3.05 | 3.03 | 2.94 | 2.87 |
| | 2.86 | 2.84 | 3.05 | 2.95 | 2.92 | 2.82 |
| | .... | .... | .... | .... | 2.80* | 2.75* |
| | .... | .... | .... | .... | 2.84* | 2.80* |
| Green tea (5) | 1.64 | 1.63 | 1.61 | 1.55 | 1.70 | 1.61 |
| | 1.65 | 1.59 | 1.69 | 1.60 | 1.66 | 1.58 |
| | .... | .... | .... | .... | 1.77 | 1.66 |
| | .... | .... | .... | .... | 1.57* | 1.52* |
| | .... | .... | .... | .... | 1.62* | 1.57* |
| Green tea (9) | 2.09† | 1.94 | 2.12 | 2.01 | 2.14 | 2.08 |
| Black tea (10) | 2.71† | 2.63 | 2.69 | 2.67 | 2.62 | 2.62 |
| Black tea (12) | 3.10† | 2.96 | 3.20 | 3.12 | 3.00 | 2.93 |
| | .... | .... | .... | .... | 3.15 | 3.03 |
| | .... | .... | .... | .... | 3.12 | 2.99 |

*Results by H. A. Lepper.

†Not purified by treatment with potassium hydroxide.

The results obtained by the proposed method are in close agreement with those obtained by the other two methods and the caffeine residues are of an equal degree of purity. The time required is very much less than in either of the other procedures.

So far as results are concerned, there is little to choose among the three methods. The Power-Chesnut method possesses a considerable advantage over the Stahlschmidt method in that it avoids the use of lead acetate. It also has a range of applicability which your referee can not claim for the Stahlschmidt procedure; for example, the referee on coffee has found the Stahlschmidt method unsatisfactory for the determination of caffeine in that substance. It seems advisable to adopt, as an official method, a procedure as widely applicable to the determination of caffeine as possible, and at the same time to include, under special subjects, any additional method of demonstrated merit and usefulness as a tentative procedure. The Stahlschmidt method with the modification made this year would be recommended as a tentative method were it not for the very satisfactory showing made by the simplified method herein proposed. This method merits further trial by a larger number of analysts.

## RECOMMENDATIONS.

It is recommended—

(1) That the modified Stahlschmidt method, as recommended last year for adoption as official for the determination of caffeine in tea, be not made official this year.

(2) That the Power-Chesnut method, page 290, be made official for the determination of caffeine in tea.

(3) That the proposed method for the determination of caffeine, page 292, be given further trial by a larger number of analysts with a view to its adoption as a tentative method.

The meeting adjourned at 5:15 p. m. for the day.

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## ANNOUNCEMENTS

Miss Nellie A. Parkinson has resigned from the Bureau of Chemistry of the United States Department of Agriculture to accept the position of Assistant to the Editor of the Journal of Industrial and Engineering Chemistry. Miss Parkinson has served for some time as associate editor of our *Journal* and is well known to most members of the association. She carries with her the best wishes of many friends for continued success in her new work.

Miss Marian E. Lapp has been selected to succeed Miss Parkinson as associate editor.

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It was with a distinct shock that we learned of the death of Dr. William Frear, which occurred at his home in State College, Pa., on January 7, 1922. Dr. Frear was one of the oldest, most active and best known of the members of this association. A biographical sketch in appreciation of his life and work will appear in a later number of *The Journal.*

Board of Editors,

R. W. Balcom, *Chairman.*
R. E. Doolittle,
R. B. Deemer,
C. B. Lipman.

---

## THIRD DAY.

# WEDNESDAY—MORNING SESSION.

## REPORT OF THE COMMITTEE ON EDITING METHODS OF ANALYSIS[1].

Your committee is pleased to report that during the past year the methods, as revised to November 1, 1919, have been published and distributed to all subscribers. The difficulties attending the publication of these methods have fallen almost entirely upon the secretary's office, but your committee has devoted considerable time to the final preparation of the manuscript for the printers and to the reading of proof. Unfortunately, the first lot of galley proof, which included the first three chapters of the methods and the greater part of Chapter XXX, the reference tables, was lost in the mails. Additional copies of the galley proof for Chapters I, II and III were later secured, but no proof for the reference tables, with the exception of those for alcohol, was received. A threatened strike of the printers also made it impossible for your committee to be furnished with copies of the page proof before final printing. However, the proof reading was looked after by J. A. MacLaughlin of Dr. Alsberg's office, and the committee desires to accord to him its appreciation and thanks for his assistance during the past year and also to Miss N. A. Parkinson who prepared the greater part of the copy for the printer but who, on account of the press of work on *The Journal*, was unable to look after the proof reading.

It is to be expected that in a book of the size of the present edition of the methods and especially with the difficulties attending its publication, some errors would occur. Fortunately, these errors are not serious in so far as any of the methods are concerned. Your committee has, however, gone over the book very carefully and made a list of the errors found, which list forms a part of this report.

Your committee also has prepared a list by chapters of the changes and additions which were made to the Official and Tentative Methods of Analysis at the 1919 meeting of the association. It will be recalled that at the meeting in November 1919 it was decided not to incorporate the changes and additions made at that meeting for the reason that the manuscript was ready for the printer and it was feared that if it were again revised the printing of the methods might be delayed. A few of the changes which could be incorporated without altering the chapter or paragraph numbers were inserted, however. These are

[1] Presented by R. E. Doolittle.

noted in the committee's report. The list of the 1919 additions and changes is submitted for the information of the members of the association and subscribers to the book of methods.

With the publication of the revised methods it would appear that the work of your committee, which was appointed at the 1914 meeting for the special purpose of revising Bureau of Chemistry Bulletin 107, has been completed. The additions and changes made to the methods, however, should be compiled each year for the information of the members of the association and the subscribers to the book of methods. To be of greatest value, this compilation should be prepared and published immediately after the close of each annual meeting. While your committee feels that the work for which it was appointed has been completed, it respectfully recommends that the association provide for the appointment of a permanent committee on editing methods of analysis whose duty it shall be to compile the additions and changes made to the official and tentative methods.

## ERRATA IN OFFICIAL AND TENTATIVE METHODS OF ANALYSIS[1].

# PREFACE

Page iii, *Line 20.*—Change initials of Dr. Gascoyne from "J. W." to "W. J."

## CONTENTS

Page x, *Line 2.*—Change "v" to "vii".
*Line 2 from bottom of page.*—Change "209" to "309".

## X. COLORING MATTER IN FOODS.

21 PROCEDURE.

*Page 141, line 6.*—Omit the word "Plum" after "Radish"; insert commas after the words "Cranberry" and "Cherry", making the line read: "Purple Grape, Cranberry, Sloe, Cherry, Plum, Radish and Red Beet are described".

22 TABLE 10.

*Page 143, line 18.*—Change "Xanthopyll" to "Xanthophyll".

BIBLIOGRAPHY.

*Reference 5.*—Change "Thrum" to "Thrun".

## XXII. FATS AND OILS.

36 HALPHEN TEST.

Change "Halpen" to "Halphen".

[1] *Assoc. Official Agr. Chemists, Methods.*, 1920.

## XXX. REFERENCE TABLES.

**1** MUNSON AND WALKER'S TABLE.

*Page 325, column 7, line 3 from bottom of page.*—Change "140.0" to "144.0".

*Page 326, column 5, line 9.*—Change "196.7" to "106.7".

*Page 328, column 9, line 22.*—Change "254.0" to "264.0".

*Page 331, column 4, line 10.*—Change "226.7" to "226.1".

*Page 331, column 5, line 23.*—Change "233.4" to "232.5".

**3** *Densities of solutions of cane sugar at 20°C.*

*Page 341, column 5, line 3 from bottom of page.*—Change "1.535791" to "1.535704".

**4** *Corrections to be applied to results obtained by* **3** *when the specific gravity is obtained at temperatures other than 20°C.*

*Page 342.*—Delete this table as it is an earlier edition of **9**, page 388.

**5** GEERLIGS' TABLE.

*Page 343, column 6, line 16.*—Change "16" to "0.0016".

*Page 343, column 9, line 10 from bottom of page.*—Change "1.7" to "0.7".

**7** ALCOHOL TABLE.

*Page 356, column 11, line 10.*—Change "81.12" to "82.12".

**8** ALCOHOL TABLE.

*Page 379, column 5, line 13 from bottom of page.*—Change "27.73" to "26.73".

**10** *Degrees Brix, specific gravity, and degrees Baumé of sugar solutions.*

*Page 389, column 2, line 25.*—Change "1.00759" to "1.00758".

*Page 393, column 2, line 14 from bottom of page.*—Change "1.17183" to "1.17185".

TABLE OF INTERNATIONAL ATOMIC WEIGHTS, 1917[1].

Insert Table of International Atomic Weights, 1917, at end of tables.

## INDEX

*Page 401, line 29.*—Change page "249" to "247".

*Page 404, line 10.*—Change page "294" to "299".

*Page 404, line 12.*—Change page "229" to "299".

*Page 406, line 34.*—Change "XV, 19" to "XV, 9, 10".

*Page 408, line 15.*—Change page "113" to "133".

*Page 408, line 10 from bottom of page.*—Page number omitted. Insert "88".

*Page 409, line 13 from bottom of page.*—Change page "15" to "13".

*Page 410, line 10 from bottom of page.*—Omit the words "alcohol" and "extracts" and insert the words "and insoluble" after the word "soluble", making the expression read "soluble and insoluble in meat".

*Page 410, line 3 from bottom of page.*—Change the word "products" to "extracts and similar products", making the expression read "insoluble, in meat extracts and similar products".

*Page 413, line 11.*—Change page "302" to "309".

*Page 417, line 21.*—Change page "215" to "216".

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[1] *J. Am. Chem. Soc.* 1916, **38**: 2220.

# 1919 CHANGES AND ADDITIONS IN OFFICIAL AND TENTATIVE METHODS OF ANALYSIS[1].

## I. FERTILIZERS.

2 PREPARATION OF SAMPLE.

The directions for sampling which appear in the close print in the second paragraph were supplemented by instructions covering the number of bags to be sampled as follows:

Take cores from not less than 10 per cent of the bags present, unless this necessitates cores from more than 20 bags, in which case take a core from 1 bag for each additional ton represented. If there are less than 100 bags, sample not less than 10 bags. In lots of less than 10 bags sample all bags.

The directions for sampling as adopted by the association, arranged according to procedure, are therefore as follows:

Each official sample sent to the laboratory shall consist of at least a pound of the material taken in the following manner: Employ a sampler that removes a core from the bag from top to bottom. Take cores from not less than 10 per cent of the bags present, unless this necessitates cores from more than 20 bags, in which case take a core from 1 bag for each additional ton represented. If there are less than 100 bags, sample not less than 10 bags. In lots of less than 10 bags, sample all bags. Pass the entire sample submitted to the analyst through a 10-mesh sieve previous to its subdivision for analysis.

## II. INORGANIC PLANT CONSTITUENTS.

The following methods for the determination of calcium and magnesium were adopted as tentative methods. These methods have been printed in the proceedings of the association[2].

### CALCIUM.—TENTATIVE.

Remove 25 cc. of the solution, representing 0.5 gram of ash, and dilute to 200 cc., add a few drops of alizarine or methyl orange and make slightly ammoniacal. Add very dilute hydrochloric acid (1 to 10) until the solution is just faintly acid, followed by 10 cc. of 0.5N hydrochloric acid and 10 cc. of 2.5% oxalic acid. Boil the solution and add, with constant stirring, 15 cc. of a saturated solution of ammonium oxalate. Continue to heat until the precipitate becomes granular. Cool and add, with constant stirring, 8 cc. of 20% sodium acetate solution, and allow to stand 12 hours. Filter, and wash with hot water until free from chlorides. Dissolve the precipitate in hot, dilute sulfuric acid and titrate with 0.1N potassium permanganate solution. In dissolving the precipitate it is best first to wash it off the paper into a beaker, and then to dissolve the portion remaining on the paper with hot, dilute sulfuric acid. (1 cc. of 0.1N $KMNO_4$ = 0.0028 gram CaO.)

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[1] *Assoc. Official Agr. Chemists, Methods*, 1920.
[2] *J. Assoc. Official Agr. Chemists*, 1921, 4: 392.

MAGNESIUM—TENTATIVE.

To the combined filtrate and washings from the calcium determination, add 25 cc. of strong nitric acid and evaporate to dryness. Take up with dilute hydrochloric acid and make to a volume of about 100 cc. Add 5 cc. of a 10% sodium citrate solution and 10 cc. of sodium hydrogen phosphate solution, or enough to precipitate all of the magnesium. Add dilute ammonium hydroxide, with constant stirring, until the solution is faintly alkaline; then add about 25 cc. of strong ammonium hydroxide and set aside in a cool place overnight. Filter and wash with 2.5% ammonium hydroxide. Dissolve the precipitate in dilute hydrochloric acid and reprecipitate as before. Allow to stand for several hours, filter and wash free of chlorides with 2.5% ammonium hydroxide solution, ignite and weigh as magnesium pyrophosphate.

## III. WATERS.

**52, 53** and **54** BARIUM.

The method for the determination of barium in waters was made an official method. (Second and final presentation of the method for action.) The method has been published in *The Journal*[1] and also in the Official and Tentative Methods of Analysis[1].

**60** and **61** BISMUTHATE METHOD.

The bismuthate method for the determination of manganese was adopted as an official method. (Second and final presentation of the method for action.)

The following method for the determination of iodine in the presence of chlorine and bromine was adopted as a tentative method. The method has been published in the proceedings[2].

IODINE IN THE PRESENCE OF CHLORINE AND BROMINE—TENTATIVE.

REAGENTS.

(a) *Sodium hydroxide and sodium carbonate solution.*—Dissolve 2.5 grams of sodium hydroxide and 2.5 grams of sodium carbonate in water and dilute to 100 cc.

(b) *Dilute sulfuric acid (1 to 10).*

(c) *Sodium hydroxide solution.*—Dissolve 4 grams of sodium hydroxide in water and dilute to 100 cc.

(d) *Potassium permanganate solution.*—Dissolve 10 grams of potassium permanganate in water and dilute to 100 cc.

(e) *0.05N sodium thiosulfate solution.*—Dissolve 12.4 grams of recrystallized sodium thiosulfate in 1 liter of water. This solution should be standardized against 0.05N potassium dichromate.

DETERMINATION.

Take such a quantity of the brine or water as will contain not more than 0.1 gram of iodine or more than 10.0 grams of total salts. Adjust the volume to 100–150 cc., add a sufficient quantity of the solution of sodium hydroxide and sodium carbonate to

[1] *J. Assoc. Official Agr. Chemists*, 1920, **4**: 86; *Assoc. Official Agr. Chemists, Methods*, 1920, 34.
[2] *Ibid.*, 1921, **4**: 380.

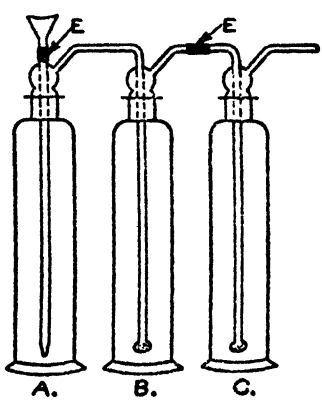
precipitate the calcium and magnesium. Boil, filter off the precipitate of calcium and magnesium, and wash with hot water; introduce the filtrate into an Erlenmeyer flask, adjust the volume to about 100 cc., neutralize with the dilute sulfuric acid and add 1 cc. of the sodium hydroxide solution. Heat to boiling, add an excess of the potassium permanganate solution, about 0.5 cc. excess, continue heating until the precipitate begins to coagulate and then allow to cool. Add sufficient 95% alcohol or hydrogen peroxide to bleach the permanganate color and set the beaker on a steam bath. When the precipitate has settled, filter and wash the precipitate with hot water. After cooling, add 1–2 grams of potassium iodide, acidify with hydrochloric acid and titrate with 0.05N thiosulfate. One-sixth of the iodine titrated represents the amount originally present. (1 cc. of 0.05N thiosulfate solution = 1.058 mg. of iodine.)

The reactions are as follows:

REACTIONS.

$$KI + 2\ KMnO_4 + H_2O = KIO_3 + 2\ KOH + 2\ MnO_2.$$
$$KIO_3 + 5\ KI + 6\ HCl = 6\ KCl + 3\ H_2O + 3\ I_2.$$
$$3\ I_2 + 6\ Na_2S_2O_3 = 6\ NaI + 3\ Na_2S_4O_6.$$

The following method for the determination of bromine in the presence of chlorine but not iodine was adopted as a tentative method. The method has been published in the proceedings[1].




A. REACTION CYLINDER.
B & C. ABSORPTION CYLINDERS.
E. RUBBER CONNECTIONS.

BROMINE IN PRESENCE OF CHLORINE BUT NOT IODINE.

REAGENTS.

(a) *Sodium sulfite and sodium carbonate solution.*—Dissolve 4 grams of sodium sulfite and 0.8 gram of sodium carbonate in water and dilute to 100 cc.

(b) *Chromium trioxide crystals.*

(c) *Hydrogen peroxide solution (3%).*

(d) *0.05N sodium thiosulfate solution.*

APPARATUS.

The apparatus used consists of 2 Dreschel gas wash bottles, high form, joined as shown in Fig. 1. An ordinary wash bottle may be substituted for *C* if desired.

DETERMINATION.

Evaporate the sample of water or brine, which should not be too acid, to dryness or nearly so. Charge the reaction cylinder *A*, Fig. 1, by introducing glass beads to a depth of about 1 inch, followed by 15 grams of solid chromium trioxide, and finally enough glass beads to fill the cylinder half full. Add 20 cc. of the sodium sulfite and sodium carbonate solution to the first absorption cylinder *B* and 5 cc. to the second *C*. Dilute each to about 200 cc. Connect the three cylinders and draw a current of air

[1] *J. Assoc. Official Agr. Chemists*, 1921, 4: 381.

through slowly. Wash the sample into the reaction cylinder with water sufficient to make about 25 cc. of solution. Aspirate until the contents of the reaction cylinder are in solution and thoroughly mixed, then discontinue, close the inlet tube with a small piece of rubber tubing and a clamp, and reduce the pressure in the apparatus slightly by suction, in order to guard against any possible escape of bromine at the ground-glass stopper. Allow to stand overnight, then aspirate with a rather strong current of air (about ½–¾ liter per minute) for 3 hours, adding 4 portions of 2 cc. each of the hydrogen peroxide solution at 30-minute intervals. Stop the aspiration and evaporate the contents of the two absorption cylinders nearly to dryness. Empty the reaction cylinder, clean, and freshly charge with glass beads and 15 grams of chromium trioxide. To the first absorption cylinder add 10 grams of potassium iodide dissolved in 200 cc. of water, and to the second 3–4 grams in a like amount of water. Connect the apparatus, draw through a slow current of air and transfer the contents of the evaporating dish to the reaction cylinder by means of the small funnel, using 25 cc. of water. Aspirate until all of the bromine is evolved (about 1 hour) and titrate the potassium iodide solution with the thiosulfate. (One cc. of 0.05N thiosulfate = 3.996 mg. of bromine.)

The reactions are as follows:

REACTIONS.

$$2\ CrO_3 + 6\ HBr = Cr_2O_3 + 3\ H_2O + 3\ Br_2.$$
$$2\ H_2CrO_4 + 3\ H_2O_2 = Cr_2O_3 + 2\ O_2 + 5\ H_2O.$$
$$Na_2SO_3 + 2\ Br + H_2O = 2\ HBr + Na_2SO_4.$$

The following method for the determination of free and albuminoid ammonia in water containing sulfide was adopted as a tentative method. The method has been published in the proceedings[1].

FREE AND ALBUMINOID AMMONIA.—TENTATIVE.

(In samples containing sulfide.)

REAGENTS.

(a) *0.5N solution of sulfuric acid.*

(b) *5N solution of sodium carbonate.*

(c) *Ammonia-free water.*

(d) *Standard ammonium chloride solution.*—Prepare as directed under **10** (c).

(e) *Nessler reagent.*—Prepare as directed under **10** (d).

(f) *Alkaline potassium permanganate solution.*—Prepare as directed under **10** (e).

DETERMINATION.

Place 500 cc. of the sample in a beaker or casserole, add 30 cc. excess of 0.5N sulfuric acid solution. Boil the solution carefully until free of sulfide (about 20 minutes). Add about 300 cc. of distilled water and 8 cc. of 5N sodium carbonate solution to a distillation flask connected as described under **11**, and distil until free from ammonia. Cool, add the cooled sample which has been freed from sulfide, and proceed as described under **11**, beginning with "Distil into 50 cc. Nessler tubes".

## IV. TANNING MATERIALS.

No changes or additions to these methods were made at the 1919 meeting.

[1] *J. Assoc. Official Agr. Chemists*, 1921, 4: 387.

## V. LEATHERS.

No changes or additions to these methods were made at the 1919 meeting.

## VI. INSECTICIDES AND FUNGICIDES.

A method for the determination of total arsenic in magnesium arsenate[1] was adopted as an official method. (First presentation of the method for adoption as official.)

A method for the determination of total arsenious oxide in magnesium arsenate[1] was adopted as an official method. (First presentation of the method for adoption as official.)

A method for the determination of total arsenious oxide in calcium arsenate[1] was adopted as an official method. (First presentation of the method for adoption as official.)

These methods should be inserted in the Official and Tentative Methods of Analysis, after **36**, in the following manner:

TOTAL ARSENIOUS OXIDE[1].—TENTATIVE.

Proceed as directed under **33**.

MAGNESIUM ARSENATE.

TOTAL ARSENIC.—TENTATIVE.

Proceed as directed under **27**.

TOTAL ARSENIOUS OXIDE.—TENTATIVE.

Proceed as directed under **33**.

## VII. FOODS AND FEEDING STUFFS.

MOISTURE.

The following method for the determination of water by drying over lime in vacuo without heat was adopted as a tentative method. The method has been printed in the proceedings[2].

Weigh 2 grams of the material into a suitable dish or crucible with a tightly fitted cover. Place in a vacuum desiccator over about 400 grams of freshly powdered ignited lime, and exhaust with a vacuum pump. After 24 hours, open the desiccator, forcing the incoming air through concentrated sulfuric acid and make the first weighing. After weighing, replace the dish in the desiccator and repeat the process until constant weight is obtained. The lime should be changed on the third or fourth day and, with very wet substances, once again near the end of the process.

The following method for the determination of water by drying over calcium carbide in vacuo without heat was adopted as a tentative method. The method has been printed in the proceedings[2].

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[1] *J. Ind. Eng. Chem.* 1916, **8**: 327.
[2] *J. Assoc. Official Agr. Chemists*, 1920, **4**: 247.

Weigh 2 grams of the material into a suitable dish or crucible with a tightly fitted cover. Place in a vacuum desiccator over about 400 grams of clean lumps of calcium carbide, and exhaust with a vacuum pump. After 24 hours, open the desiccator, forcing the incoming air through concentrated sulfuric acid and make the first weighing. After weighing, replace the dish in the desiccator and repeat the process until constant weight is obtained. The calcium carbide should be changed on the third or fourth day and, with very wet substances, once again near the end of the process.

## VIII. SACCHARINE PRODUCTS.

1 PREPARATION OF SAMPLE.

Directions for the preparation of samples of raw sugar for analysis were adopted by adding to 1 (c) the following:

In the case of raw sugars, mix thoroughly, and in the shortest possible time, on a watch glass with a spatula; when lumps are present, reduce them with a glass or iron rolling pin; or mix thoroughly in the shortest possible time in a large, clean, dry mortar, using a pestle to reduce lumps if present.

The following temperature formula for correcting the polarization of raw sugars to 20°C. was adopted:

Normal raw cane sugar polarizations made at other temperatures than the standard temperature of 20°C. may be calculated to the polarization at 20°C. by the following formula:

$P^{20} = P^t + 0.0015\ (P^t - 80)\ (t - 20)$, in which

$P^t$ = the polarization at which temperature is read; and

$t$ = the temperature at which polarization is read.

When the percentage of levulose in the sugar is known, the following formula should be used:

$P^{20} = P^t + 0.0003°S\ (t - 20) - 0.00812°L\ (t - 20)$ in which

$P^t$ = the polarization at which temperature is read;

$t$ = the temperature at which polarization is read;

$S$ = the percentage of sucrose; and

$L$ = the percentage of levulose.

The Baumé scale[1] (Modulus 145) of the Bureau of Standards was adopted as the official Baumé scale of the association and all Baumé tables and references thereto not in accordance with this scale were eliminated from the methods of the association. The Committee on Editing Methods of Analysis was authorized to make such changes in the text of this chapter as were necessary to make this change effective. These changes are as follows:

5 *By Means of a Spindle.—Official.*

This determination has been changed to read as follows:

The density of juices, sirups, etc., is most conveniently determined by means of the Brix hydrometer. For rough work, or where less accuracy is desired, the Baumé

[1] U. S. Bur. Standards Circ. 44: (1918), 151.

hydrometer may be used. A table for the comparison of specific gravities at $\frac{20°C.}{4°}$ and $\frac{20°C.}{20°}$, degrees Brix (per cent by weight of sucrose) and degrees Baumé, is given under **XXX**, Table 10. The Brix spindle should be graduated to tenths and the range of each individual spindle should be as limited as possible. The solution should be as nearly as practicable of the same temperature as the air at the time of reading, and, if the variation from the temperature of the graduation of the spindle amounts to more than 1°, a correction must be applied according to the table under **XXX, 9**.

Before taking the density of a juice, allow it to stand in the cylinder until all air bubbles have escaped and until all fatty or waxy matter has come to the surface and been skimmed off. The cylinder should be large enough in diameter to allow the hydrometer to come to rest without touching the sides. A table of specific gravities at $\frac{20°C.}{4°}$ and of per cent by weight of sucrose, is given under **XXX, 3**. A table for comparison of specific gravities at $\frac{20°C.}{4°}$ and $\frac{20°C.}{20°}$, degrees Baumé (Modulus 145) and degrees Brix (per cent by weight of sucrose) is given under **XXX, 10**.

If the sample is too dense to determine the density directly, dilute a weighed portion with a weighed quantity of water, or dissolve a weighed portion and dilute to a known volume with water.

In the first instance the per cent of total solids is calculated by the following formula:

Per cent of solids in the undiluted material $= \frac{WS}{w}$ in which

S = per cent of solids in the diluted material;
W = weight of the diluted material; and
w = weight of sample taken for dilution.

When the dilution is made to a definite volume, the following formula is to be used:

Per cent of solids in the undiluted material $= \frac{VDS}{W}$ in which

V = volume of the diluted solution at a given temperature;
D = specific gravity of the diluted solution at the same temperature;
S = per cent of solids in the diluted solution at the same temperature; and
W = weight of the sample taken for dilution at the same temperature.

If the spindle reading be made at any other temperature than 20°C. the results should be corrected as directed under **XXX, 9**.

**6** TABLE 7.

Eliminate this table and substitute therefor **XXX, 9**.

**7 (b)**.—This paragraph has been corrected to read as follows:

(b) *By specific gravity at* $\frac{20°C.}{20°}$.—Proceed as directed under (a), the determinations of specific gravity being made at $\frac{20°C}{20°}$ instead of $\frac{20°C.}{4°}$. Ascertain the corresponding per cent by weight of sucrose from **XXX, 10**.

## MAPLE PRODUCTS.

The following method for determination of the Canadian lead number was adopted as a tentative method. The method has been printed in the proceedings[1].

### CANADIAN LEAD NUMBER.—TENTATIVE.

#### REAGENTS.

*Standard basic lead acetate solution.*—Boil 280 grams of dry basic lead acetate [**VII, 13 (c)**] with 500 cc. of water. When solution is complete except for a slight sediment, pour off into a beaker or allow to cool in dish and dilute with recently boiled water to a density of 1.25 at 20°C.

[1] *J. Assoc. Official Agr. Chemists*, 1921, **4**: 437.

DETERMINATION.

Weigh the quantity of the sirup prepared as directed under **50**, containing 25 grams of dry matter, transfer to a 100 cc. volumetric flask, cool to 20°C. and make up to the mark. Pipet 20 cc. into a large test tube, add 2 cc. of the standard basic lead acetate solution and mix. Allow to stand for 2 hours. Filter on a tared Gooch, having an asbestos mat at least 3 mm. thick, wash four or five times with boiling water, dry at 100°C. and weigh. Multiply the weight of the dry precipitate by 20.

The following method for the determination of conductivity value was adopted as a tentative method. This method has been printed in the proceedings[1].

CONDUCTIVITY VALUE.—TENTATIVE.

*Determination of the cell constant.*—Prepare 0.1, 0.05 and 0.01N potassium chloride solutions by dissolving respectively, 7.4560, 1.4912 and 0.7456 grams of pure, ignited potassium chloride in water and making up to 1 liter at 18°C. In a 100 cc. beaker place 60 cc. of the 0.01N solution, insert a Van Zoeren or other dipping electrode, bring to 25°C. and measure the electrical resistance. Multiply the number of ohms found by 141.2. Rinse the electrode and beaker with the 0.05N solution, add 60 cc. of this solution, measure its resistance at 25°C. and multiply by 276.8. Rinse with the 0.1N solution, add 60 cc. of this solution, measure its resistance at 25°C. and multiply by 1289. Average the three results (which should agree within 1 per cent) and multiply by $10^5$.

DETERMINATION.

Weigh the quantity of sirup containing 22 grams of dry matter. Transfer to a 100 cc. volumetric flask with warm water, cool and make up to the mark. Measure 60 cc. of the solution into a 100 cc. beaker, insert a Van Zoeren or other dipping electrode, bring to 25°C. (±0.1°) and measure the electrical resistance. Divide the constant of the cell by the observed number of ohms and multiply the result by $10^5$.

## IX. FOOD PRESERVATIVES.

No changes or additions to these methods were made at the 1919 meeting.

## X. COLORING MATTERS IN FOODS.

No changes or additions to these methods were made at the 1919 meeting.

## XI. METALS IN FOODS.

**5** *Gravimetric Method.*

Adopted as an official method. (First presentation of the method for action as official.)

**6** *and* **7** *Volumetric Method.*

Adopted as an official method. (First presentation of the method for action as official.)

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[1] *J. Assoc. Official Agr. Chemists*, 1921, 4: 435.

## XII. FRUITS AND FRUIT PRODUCTS.

No changes or additions to these methods were made at the 1919 meeting.

## XIII. CANNED VEGETABLES.

No changes or additions to these methods were made at the 1919 meeting.

## XIV. CEREAL FOODS.

No changes or additions to these methods were made at the 1919 meeting.

## XV. WINES.

No changes or additions to these methods were made at the 1919 meeting.

## XVI. DISTILLED LIQUORS.

No changes or additions to these methods were made at the 1919 meeting.

## XVII. BEERS.

No changes or additions to these methods were made at the 1919 meeting.

## XVIII. VINEGARS.

1 PHYSICAL EXAMINATION.

Adopted as an official method. (First presentation of the method for adoption as official.)

4 ALCOHOL.

Adopted as an official method. (First presentation of the method for adoption as official.)

## XIX. FLAVORING EXTRACTS.

The following method for the determination of alcohol in lemon and orange extracts[1] was adopted as an alternate official method. (First presentation of the method for adoption as official.)

### ALCOHOL.

*Method II.—Tentative.*

(Applicable only to extracts consisting of oil, alcohol and water.)

Let $S$ represent the specific gravity of the extract at 20°C./4° as determined under **17**; $O$ the specific gravity of the oil and $p$ the per cent of oil found. Then 100-$p$ will be the per cent of the water-alcohol solution, the specific gravity of which, represented by $P$, is calculated as follows:

$$S=\frac{Op+P(100-p)}{100}, \text{ whence } P=\frac{100S-Op}{100-p}.$$

[1] *J. Ind. Eng. Chem.*, 1909, **1**: 94.

The value of $E$, the alcohol equivalent of $P$, is obtained from **XXX, 1** and gives the per cent of alcohol in the alcohol-water solution. To find the per cent of alcohol in the extract, apply the following formula:

Per cent by volume of alcohol in the extract $= E\ (1-\frac{P}{100})$.

The value of $O$ for lemon extract may be taken as 0.85 and for orange extract as 0.84.

## XX. MEAT AND MEAT PRODUCTS.

### Page 210, NITRATES.

This heading was changed to read "Nitrates and Nitrites (calculated as sodium nitrate)" and the method has been changed to provide that the results be calculated to sodium nitrate instead of potassium nitrate. The last sentence of **11** has accordingly been changed to read as follows:

One cc. of nitric oxide at 0°C. and 760 mm. pressure is equivalent to 0.0037935 gram of sodium nitrate.

The following method for the determination of sugar was adopted as a tentative method to be substituted for the former tentative method[1]. This substitution has been made and the method printed in the 1920 edition of the methods[2].

### SUGAR.—TENTATIVE.

**19** REAGENT.

*Phosphotungstic acid solution.*—Dissolve 100 grams of phosphotungstic acid in water and dilute to 100 cc.

**20** DETERMINATION.

Weigh 100 grams of the finely ground sample into a 600 cc. beaker, add 200 cc. of water, heat to boiling and boil gently for 5 minutes. Stir the contents of the beaker frequently during this and subsequent extractions to prevent lumping. (When several samples are extracted at the same time a mechanical stirring device is practically a necessity.) Remove the beaker from the flame, allow the insoluble matter to settle and decant the clear liquid on an asbestos mat in a 4-inch funnel. Filter with the aid of suction. Add 150 cc. of hot water to the residue in the beaker, boil gently for 5 minutes, let settle and decant the clear liquid as above. Repeat the operation and finally transfer the contents of the beaker to the funnel, wash with 150-200 cc. of hot water and press the meat residue as dry as possible. Transfer the contents of the filter flask to an evaporating dish and evaporate on a steam bath to a volume of about 25 cc., but not to dryness. Transfer the extract to a 100 cc. volumetric flask, taking care that the volume of liquid does not exceed 60 cc. Add 25-35 cc. of the phosphotungstic acid solution, shake vigorously, let stand a few minutes for gas bubbles to rise to the surface, make to volume, shake and either filter or centrifugalize. The use of a centrifuge is to be preferred since thereby a large volume of liquid is obtained. Test a portion of the filtrate with dry phosphotungstic acid for complete precipitation. If an appreciable precipitate forms, take an aliquot of the filtrate, add 5—10 cc. of the phospho-

[1] *Assoc. Official Agr. Chemists, Methods*, 1916, 278.
[2] *Ibid.*, 1920, 213.

tungstic acid solution, make to volume, filter and test the filtrate for complete precipitation. The filtrate should also show not more than a slight reaction for creatinin by Jaffe's test[1].

Transfer 50 cc. of the clarified extract to a 100 cc. volumetric flask, add 5 cc. of concentrated hydrochloric acid and invert the solution as directed under **VII, 14.** Cool the solution, neutralize to litmus, cool, make to volume and filter. To the filtrate add sufficient dry powdered potassium chloride to precipitate the excess of phosphotungstic acid, filter, test the filtrate for complete precipitation, and determine the reducing sugar, as directed under **VII, 25,** ascertaining the amount of reduced copper, as directed under **VII, 29.** Calculate the total sugar as dextrose.

If, when the clarified meat extract is boiled with Fehling's solution an abnormal reduction is obtained, *i. e.*, the solution turns yellow, brown, green or muddy in appearance instead of reddish-blue, the determination should be discarded, since incomplete precipitation of the nitrogenous compounds, due to the use of insufficient phosphotungstic acid, is indicated.

## XXI. DAIRY PRODUCTS.

**65** *Roese-Gottlieb Method.*

The Roese-Gottlieb method for the determination of fat in plain ice cream, a tentative method, was made official. (Second presentation of the method for adoption as official.) The method is included in the referee's report for 1917 and has been published in the 1920 edition of the methods[2].

## XXII. FATS AND OILS.

The Hübl Method[3] for the determination of the iodine absorption number was dropped from the official methods. This method has not been included in the 1920 edition of the methods. The Wijs method for the determination of the iodine absorption number[4] was adopted as a tentative method. This method has been inserted **17** and **18,** in the 1920 edition of the methods.

A recommendation "That all reports of iodine absorption number should specify the method used" was adopted. This sentence has accordingly been inserted in parenthesis following the heading "Iodine Absorption Method" on page 244 of the 1920 edition of the methods.

## XXIII. SPICES AND OTHER CONDIMENTS.

**17** VOLATILE OIL IN MUSTARD SEED.

Adopted as an official method. (First presentation of the method for adoption as official.) This method is printed in the 1920 edition of the methods.

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[1] *C. A.* 1910, **4**: 218.
[2] *Assoc. Official Agr. Chemists, Methods*, 1920, 236.
[3] *Ibid.*, 1916, 304.
[4] *Ibid.*, 1920, 245.

## XXIV. CACAO PRODUCTS.

No changes or additions to these methods were made at the 1919 meeting.

## XXV. COFFEES.

14 THE FENDLER-STÜBER METHOD.—TENTATIVE.

Certain modifications of the Fendler-Stüber method for the determination of caffeine were adopted, the modified method to remain as a tentative method. The method, in its modified form, has been printed in the proceedings[1].

15 MODIFIED STAHLSCHMIDT METHOD.—TENTATIVE.

*Page 271, line 5.*—The expression "75°C." has been changed to "100°C."

This change provides that the final drying of the caffeine crystals shall be at 100°C. instead of 75°C. The method as modified has not been published.

## XXVI. TEAS.

4 WATER EXTRACT.—TENTATIVE.

A modification of the former tentative method for the determination of water extract in teas[2] was adopted as a tentative method. The method has been published in the proceedings[3].

14 CAFFEINE.

The modified Stahlschmidt method for the determination of caffeine in teas was changed to provide that the final drying of the caffeine crystals be made at 100°C. instead of 75°C. and the method as changed was adopted as an official method. (First presentation of the method for adoption as official.) This necessitates the change in the Chapter on Coffees[4].

## XXVII. BAKING POWDERS AND BAKING CHEMICALS.

The Wagner-Ross method for the determination of fluorids in baking powders and baking powder ingredients was adopted as a tentative method. The method is as follows:

REAGENTS.

(a) *Anhydrous copper sulfate.*

(b) *Ground quartz or sand.*—Purify by successive digestions with sulfuric acid and aqua regia, wash with water and dry.

(c) *98.5% sulfuric acid.*—Prepare by boiling C. P. concentrated sulfuric acid in an open vessel for about 20 minutes.

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[1] *J. Assoc. Official Agr. Chemists*, 1921, 4: 533.
[2] *Assoc. Official Agr. Chemists, Methods*, 1916, 335.
[3] *J. Assoc. Official Agr. Chemists*, 1921, 4: 537.
[4] *Assoc. Official Agr. Chemists, Methods*, 1920, 271.

(d) *10% solution of silver sulfate in 98.5% sulfuric acid.*—Dissolve 10 grams of silver sulfate, which has been ignited with an excess of sulfuric acid to drive off any volatile acids present, in 100 cc. of the 98.5% sulfuric acid.

(e) *Saturated solution of dry chromic acid in 98.5% sulfuric acid.*

(f) *Standard sodium hydroxide solution.*—Approximately 0.1N solution, preferably standardized against chemically pure sodium fluosilicate.

(g) *Standard hydrochloric acid solution.*—Approximately 0.1N solution carefully standardized against the standard sodium hydroxide solution.

APPARATUS.

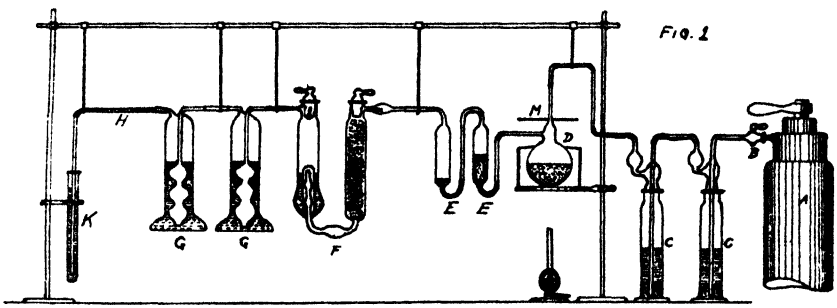


FIG. 1. APPARATUS FOR DETERMINATION OF FLUORIDS.

The apparatus required (Fig. 1) consists of a cylinder (*A*) of compressed carbon dioxide or nitrogen, preferably the latter, fitted with a reducing valve (*B*), or other safety device, for regulating the flow of gas; a drying train consisting of two wash bottles (*C*) and (*C*), containing concentrated sulfuric acid for washing the gas used; a 250 cc. generating flask (*D*), of pyrex glass, in which the sample is placed and which is provided with two washing traps (*E*) and (*E*), both containing the 98.5% sulfuric acid, the one next the generating flask being half full while the acid in the other is just sufficient to make a seal; a Schmitz tube (*F*), containing the 10% solution of silver nitrate in 98.5% sulfuric acid in the bulbed arm and glass beads (about 5 mm. in diameter) in the other arm; two Bowen bulbs (*G*) and (*G*), each containing the saturated solution of chromic acid in 98.5% sulfuric acid; a straight glass tube (*H*), 5 mm. in diameter and 14 cm. in length, filled with glass wool; a second glass tube of the same diameter and of sufficient length to extend to the bottom of the absorption tube (*K*), which is a large test tube (25 cm. by 18 cm.) containing about 50 cc. of water and, for materials relatively low in fluorine, an additional 10 cc. of the standard hydrochloric acid solution to lessen the oxidation of any sulfur dioxide that may pass into the absorption solution. The apparatus is connected as shown in Fig. 1. On account of the pressure that must be generated in the apparatus to produce a flow of gas against the head of sulfuric acid great care must be taken in assembling to make all joints tight. The ends of the glass tubing must be brought together and the rubber tubing, which must be a good grade of heavy-walled gum tubing, covered with shellac. All glass stop-cocks should be paraffined. A mantle of asbestos board, (*M*), should be placed over the neck of the generating flask to prevent burning of the rubber connection at the top of the flask. It is imperative that all parts of the apparatus be perfectly dry before use, and that the solutions of concentrated sulfuric acid be protected against absorption of moisture from the air.

### PRELIMINARY TREATMENT OF SAMPLE.

Remove all organic matter by burning. This is essential. It is carried out on the weighed portion of the sample taken for analysis by making alkaline with sodium carbonate and igniting in a muffle furnace at a temperature below redness (not to exceed 240°C.) to a white or nearly white ash. In the case of baking powder, add sufficient water to complete the reaction between the acid and bicarbonate constitutents, evaporate to dryness on a steam bath and ignite to a white ash as directed above.

### DETERMINATION.

Make a preliminary examination, using 10–20 grams of the sample to determine the approximate quantity of fluorine present.

Mix the ignited residue (organic matter removed and thoroughly dried) of a weighed sample containing 0.001–0.1 gram of fluorine with 0.5 gram of the powdered silica and 5 grams of the anhydrous copper sulfate. Transfer the mixture into the generating flask (*A*), (Fig. 1), the traps (*E*) and (*E*) having been filled by suction with the requisite quantity of 98.5% sulfuric acid without drawing any of the acid into the flask itself. Connect the generating flask with the Schmitz tube. Then add 50 cc. of water to the absorption tube (*K*), and, if the fluorine content of the sample is low also, add 10 cc. of the standard hydrochloric acid solution. Connect the absorption tube with the apparatus and then add 50–75 cc. of the 98.5% sulfuric acid to the generating flask which contains the sample mixed with the powdered silica and copper sulfate and quickly connect it with the source of carbon dioxide (or nitrogen). Adjust the flow of gas from the cylinder so as to give a rate of about 2 or 3 bubbles per second and maintain this rate throughout the determination. Shake the flask until the contents are well mixed and then heat gradually to boiling. At this point in the determination a white scum, indicating fluorine, will appear on the inside of the flask. Adjust the flame under the flask so that the condensing sulfuric acid will wash this scum freely and completely into the first trap, taking care to avoid heating so strongly that white fumes will be evolved in noticeable quantity or the acid in the first trap made to boil. Continue the boiling until the first trap is completely filled, about 30 minutes. Remove the flame, taking particular care to regulate the flow of gas so that the relatively cool acid in the traps does not flow back into the flask. Adjust the valve in the gas cylinder so as to continue a uniform flow of gas at the rate of 2 or 3 bubbles per minute for 30 minutes in order to wash all silicon fluoride into the absorption tube. Remove the latter, wash its contents into a beaker, cover with a watch glass and boil gently 10–15 minutes to expel dissolved gases. The operation of transferring the absorption solution should consume as little time as possible as any prolonged exposure to air before boiling permits the oxidation of any sulfur dioxide in solution. Cool the solution to room temperature and titrate with the standard sodium hydroxide solution, using phenolphthalein as indicator. Deduct the alkali equivalent of the standard acid added to the absorption solution and calculate the fluorine present in the sample taken. The reaction occurring is represented by the following equation:

$$H_2SiF_6 + 6\ NaOH = 6\ NaF + 2H_2O + H_4SiO_4.$$

After the solution has been titrated test for the presence of sulfates by the addition of a little barium chloride solution. If the determination has been properly carried out little or no sulfates should be present. If sulfates are present determine quantitatively by precipitation with barium chloride[1] and correct the total standard sodium hydroxide solution used in the original titration by the equivalent of sodium hydroxide for the quantity of sulfuric acid present in the titrating solution.

[1] *Assoc. Official Agr. Chemists, Methods*, 1920, 18.

## XXVIII. DRUGS.

31 STRYCHNINE IN LIQUIDS.

The following addition was made to the tentative method for the determination of strychnine in liquids:

Check the weight of the strychnine by dissolving the residue in neutral alcohol, adding an excess of 0.1N sulfuric acid, and titrating back with 0.02N potassium hydroxide, using methyl red as the indicator. One cc. of 0.1N sulfuric acid is equivalent to 0.0334 gram of strychnine and 0.0428 gram of strychnine sulfate. The U. S. P. factor for strychnine to strychnine sulfate is 1.2815.

32 STRYCHNINE IN TABLETS.

The following addition was made to the tentative method for the determination of strychnine in tablets:

Check the weight of the strychnine by dissolving the residue in neutral alcohol, adding an excess of 0.1N sulfuric acid, and titrating back with 0.02N potassium hydroxide, using methyl red as the indicator. One cc. of 0.1N sulfuric acid is equivalent to 0.0334 gram of strychnine and 0.0428 gram of strychnine sulfate. The U. S. P. factor for strychnine to strychnine sulfate is 1.2815.

## XXIX. SOILS.

No changes or additions to these methods were made at the 1919 meeting.

## XXX. REFERENCE TABLES.

No changes or additions to these tables were made at the 1919 meeting.

Respectfully submitted,

R. E. DOOLITTLE, W. H. MACINTIRE,
A. J. PATTEN, J. W. SALE,
B. B. ROSS, G. W. HOOVER.

*Committee on Editing Methods of Analysis.*

It was moved, seconded and adopted that the report be accepted, the committee discharged and that the chair appoint a Committee on Editing Methods of Analysis, consisting of six members, to serve for a period of five years. The chair accordingly reappointed the same committee to serve for a period of five years.

## REPORT OF COMMITTEE ON QUARTZ PLATES STANDARDIZATION AND NORMAL WEIGHT.

By Frederick Bates (Bureau of Standards, Washington, D. C.), *Chairman.*

Your committee, consisting of Frederick Bates, chairman, C. A. Browne and F. W. Zerban, was appointed as a result of the 1919 report of the referee on sugar[1] by the late A. Hugh Bryan. The subsequent illness and death of Mr. Bryan made it practically impossible for the committee to get any satisfactory results on the matters involved in time to present them at this meeting. It is therefore recommended that the committee be continued for another year.

Adopted.

## REPORT OF COMMITTEE ON METHODS OF SAMPLING FERTILIZERS TO COOPERATE WITH A SIMILAR COMMITTEE OF THE AMERICAN CHEMICAL SOCIETY[2].

The work of the committee was completed and reported last year[3]. The committee has nothing further to recommend at this time except a repetition of the 1919 recommendations, which are as follows, and that the committee be discharged.

### RECOMMENDATIONS.

It is recommended—

(1) That a sampler be used that removes a core from the bag from top to bottom.

(2) That at least a pound of the material should constitute each official sample sent to headquarters.

(3) That the entire sample submitted to the chemist be passed through a 10-mesh sieve previous to its subdivision for analysis.

(4) That cores shall be taken from not less than 10 per cent of the bags present, unless this necessitates cores from more than 20 bags, in which case a core shall be taken from 1 bag from each additional ton represented. If there are less than 100 bags, not less than 10 bags shall be sampled, provided that in lots of less than 10 bags all bags shall be sampled.

Respectfully submitted,

C. H. Jones,
E. G. Proulx,
B. F. Robertson.
*Committee on Methods of Sampling Fertilizers to Cooperate with a Similar Committee of the American Chemical Society.*

Adopted.

[1] *J. Assoc. Official Agr. Chemists*, 1921, 4: 321.
[2] Presented by C. H. Jones.
[3] *J. Assoc. Official Agr. Chemists*, 1921, 4: 594.

# REPORT OF COMMITTEE ON THE REVISION OF METHODS OF SOIL ANALYSIS[1].

By C. B. LIPMAN (Agricultural Experiment Station, Berkeley, Calif.), *Chairman.*

The gigantic strides which have been taken by chemists in recent years in their progress on soil studies and the relation of soils to plants, have rendered necessary very material revision, not only of the actual methods of analysis which have been in vogue in the past, but also of the point of view and conceptions of the soil chemist with reference to the value and the validity of the procedure of soil analysis itself. This committee has, therefore, tried to bear in mind these important advances, and to revise the methods of soil analysis as much as possible in accordance therewith. It must be obvious to all chemists, and particularly to all soil chemists, that the difficulties in the path of making a perfect revision are very numerous and, in some cases, almost insuperable. Your committee has, however, done the best that it could in the light of the knowledge at hand.

Three years have passed since the committee had an opportunity to give detailed consideration to this report and to the methods of soil analysis. Much, indeed very much, has transpired since, which will make it necessary to suggest to the association many further far-reaching revisions and additions. It is hoped, therefore, that the committee will be continued and that it will be requested to present to the next meeting of the association a new statement of the revised methods of soil analysis.

Adopted.

It was moved, seconded and adopted that the committee be continued and that someone be appointed to fill the vacancy caused by the resignation of E. C. Shorey.

[1] Presented by W. H. MacIntire.

# REPORT OF COMMITTEE ON VEGETATION TESTS ON THE AVAILABILITY OF PHOSPHORIC ACID IN BASIC SLAG.

By H. D. HASKINS (Agricultural Experiment Station, Amherst, Mass.), *Chairman.*

Your committee regrets its inability to make a final report on the results of cooperative field and pot experiments with basic slag phosphate. In apologizing for this apparent lethargy, the present chairman would point out the great difficulty in obtaining reliable data in a short time on the activity of different phosphates through the medium of field experiments. On fields which are not noticeably deficient in phosphorus compounds, several years of preliminary experiments are necessary in fitting the soil for a final test. In other words, a soil must be exhausted in active phosphoric acid compounds, as well as abundantly supplied with all other necessary plant food constituents, before it can furnish reliable data as to the phosphoric acid availability of the different phosphates employed in the experiment. The same is true, of course, in pot experiments, although many times a limited amount of soil exhausted in phosphoric acid may be secured from some local experimental field. Then, too, during the past several years many agricultural experiment station workers have been engaged in the business of war, and it has been impossible to carry on many activities outside of the regular routine of station work.

It is apparent from a somewhat hasty examination of the data at hand that final reports have been received from the several experimenters who undertook this cooperative work. Nine pot and five field experiments have been conducted in various parts of the country and your committee is of the opinion that from some of the pot work at least very definite and positive results will be secured as to the activity of the phosphoric acid in basic slag phosphates. In conclusion, your committee asks an extension of time for another year in order to formulate a final report.

Adopted.

It was moved, seconded and adopted that the committee be continued.

The meeting adjourned at 1 p. m. to reconvene at 2 p. m.

## THIRD DAY

# WEDNESDAY—AFTERNOON SESSION.

## REPORT OF SECRETARY-TREASURER FOR

By C. L. ALSBERG* (Bureau of Chemistry,

### RECEIPTS.

| | | |
|---|---|---|
| 1919 | | |
| Nov. 15 | Bank balance | $550.71 |
| | Dues from 2 institutions (Alabama Polytechnic Institute and San Francisco Department of Public Health) received too late to include in report for 1919 | 10.00 |
| | Dues for the year 1920 from 52 Federal, State, Municipal and Canadian organizations | 258.00† |
| | Reimbursement checks drawn on Secretary-Treasurer account for *This Journal* | 762.48 |
| | Check No. 75 (Feb. 21, 1917) on which payment was stopped | 0.16 |
| 1920 | | |
| July 13 | Transferred from Metropolitan National Bank to Commercial National Bank | 203.25 |
| | | $1784.60 |

* Present address, Food Research Institute, Stanford University, Calif.
†Two dollars transferred from Journal account to complete payment for one institution.

THE YEAR ENDING NOVEMBER 17, 1920.

Washington, D. C.), *Secretary-Treasurer.*

DISBURSEMENTS.

| 1919 | | Amt. | Check No. |
|---|---|---|---|
| Nov. 20 | J. S. Hodges, perforating 500 cooperative circulars | $ 0.80 | 123 |
| Nov. 20 | Tips, New Willard Hotel, for 1919 meeting | 30.50 | 124 |
| Nov. 20 | Car fare, phone calls, 1919 meeting | 2.20 | 125 |
| Nov. 21 | Postage | 15.00 | 126 |
| Nov. 25 | Chas. G. Stott & Co., 1500 window envelopes | 8.28 | 127 |
| Nov. 25 | 5000 special request envelopes (2 cent) | | |
| | 5000 special request envelopes (1 cent) | 185.40 | 128 |
| Nov. 28 | Ware Bros. Company, two ¼-page ads in the *American Fertilizer* | 50.00 | 129 |
| Dec. 2 | American Chemical Society, ¼-page ad in December, 1919 issue *J. Ind. Eng. Chem.* | 19.25 | 130 |
| Dec. 2 | Byron S. Adams, printing 1500 window envelopes | 3.75 | 131 |
| Dec. 3 | Postage | 15.00 | 132 |
| Dec. 5 | American Chemical Society, ¼-page ad in *J. Am. Chem. Soc.* | 9.16 | 133 |
| 1920 | | | |
| Jan. 2 | Postage | 50.00 | 134 |
| Jan. 2 | Post Office box rent for quarter ending March 31, 1920 | 2.00 | 135 |
| Jan. 6 | Chas. G. Stott & Co., printing 2000 letterheads, alphabetical index | 13.50 | 136 |
| Jan. 10 | The Science Press, ¼-page ad in November 7 and 14, 1920 issues | 10.80 | 137 |
| Jan. 10 | American Chemical Society, ¼-page ad in January, 1920 issue of *J. Ind. Eng. Chem.* | 19.25 | 138 |
| Jan. 20 | Hay Rubber Stamp Company, cushion stamp | 1.15 | 139 |
| Jan. 17 | American Chemical Society, ¼-page ad in January 1920 issue *J. Am. Chem. Soc.* | 9.16 | 140 |
| Mar. 24 | Post Office box rent for quarter ending June 30, 1920 | 2.00 | 141 |
| Mar. 29 | Postage | 50.00 | 142 |
| Apr. 9 | Chas. G. Stott & Co., printing 500 letterheads | 29.22 | 143 |
| Apr. 17 | Williams & Wilkins Co., ad in *J. Biol. Chem.* | 8.00 | 144 |
| May 21 | Chas. G. Stott & Co., paper | 4.95 | 145 |
| May 21 | A. Zichtl & Co., binding Methods and Vols. I and II *This Journal* | 7.25 | 146 |
| June 22 | C. L. Alsberg, transfer of account | 203.25 | 147 |
| July 14 | Post Office box rent for quarter ending September 30, 1920 | 2.00 | 1 |
| July 14 | Postage, 3000 one-cent stamps, 1500 two-cent stamps, 100 three-cent stamps, 50 special delivery stamps | 68.00 | 2 |
| Nov. 2 | Byron S. Adams, printing 1000 programs, 1920 meeting | 39.25 | 3 |
| Nov. 11 | Postage | 4.00 | 148 |
| Nov. 12 | Bank balance | 921.48 | |
| | | $1784.60 | |

The undersigned committee has examined the books of the Secretary-Treasurer and finds them correct.

Respectfully submitted,

ANDREW J. PATTEN,

H. H. HANSON.

*Auditing Committee.*

Adopted.

# FINANCIAL REPORT ON THE JOURNAL

By C. L. ALSBERG* (Bureau of Chemistry,

## RECEIPTS.

| | | | |
|---|---|---|---|
| 1915 | | | |
| Nov. 1 | Bank balance | | $ 569.48 |
| Dec. 31 | Interest | | 2.92 |
| | Checks and cash received | 7,638.28 | |
| | Less exchange on foreign notes | 19.01 | |
| | | | 7,619.27 |
| | Transfer of account from the National Metropolitan Bank to the American National Bank | | 1,289.68 |
| | | | $9,481.35 |

*Present address, Food Research Institute, Stanford University, Calif.

AND BOOK OF METHODS.

Washington, D. C.), *Chairman, Board of Editors.*

DISBURSEMENTS.

| | | Amt. | Check No. |
|---|---|---|---|
| 1915 | | | |
| Dec. 20 | H. A. Freeman, refund for excess payment on subscription .$ | 1.00 | 12 |
| 1916 | | | |
| Jan. 14 | N. A. Parkinson, reimbursement 2 trips to Baltimore | 3.30 | 13 |
| Jan. 29 | Williams & Wilkins Company, Baltimore, Md. | 723.35 | 14 |
| Feb. 2 | Williams & Wilkins Company, Baltimore, Md., Subscription of Eastman Kodak Company for which M. O. was deposited in bank | 5.00 | 15 |
| Apr. 18 | Chas. G. Stott & Co., printing 500 letterheads | 4.50 | 16 |
| Apr. 27 | Telegram to E. B. Passano, Baltimore, Md. | .26 | 17 |
| May 8 | Postage | 2.00 | 18 |
| May 13 | Postage | 3.00 | 19 |
| June 6 | C. L. Alsberg, telegram | .46 | 20 |
| June 30 | C. L. Alsberg, telegram | .54 | 21 |
| July 31 | R. L. Emerson, postage | 1.00 | 22 |
| Aug. 1 | F. C. Blanck, postage | .36 | 23 |
| Sep. 25 | Williams & Wilkins Company, subscription of Institute für Zuker Industrie | 5.00 | 24 |
| 1917 | | | |
| Jan. 24 | Williams & Wilkins Company, subscription of A. L. Cavalcanti | 15.50 | 25 |
| 1920 | | | |
| Feb. 13 | Payment for Volume I, No. 1, sold Azor Thurston | 1.25 | 1 |
| Mar. 4 | Industrial Printing Co. on account printing Volume III, No. 2 | 250.00 | 2 |
| Mar. 24 | Industrial Printing Co., on account printing Volume III, No. 2 | 250.00 | 3 |
| Apr. 14 | V. S. Bentley, refund on subscription | 3.75 | 4 |
| Apr. 14 | Raymond Wells, refund on subscription | 10.00 | 5 |
| Apr. 24 | Industrial Printing Co., on account Volume III, No. 2 | 250.00 | 7 |
| May 3 | Expenses N. A. Parkinson, 2 trips to Baltimore | 9.50 | 8 |
| June 2 | The Duplicating Office, 500 copies of instructions to referees | 7.10 | 10 |
| June 22 | C. L. Alsberg, transfer of account | 1,289.68 | 11 |
| July 3 | Industrial Printing Co., to complete payment on Volume III, No. 2 | 313.25 | 1 |
| July 3 | N. A. Parkinson, expenses trip to Baltimore | 5.65 | 2 |
| July 14 | Postmaster, 10,000 two-cent window envelopes | 243.80 | 3 |
| July 15 | The Duplicating Office, 5000 letterheads, 3000 circular letters | 45.15 | 4 |
| July 21 | G. E. Stechert & Co., commission on subscriptions | 3.20 | 6 |
| July 24 | Joe Cohen, affidavits for J. A. MacLaughlin | 2.00 | 7 |
| July 30 | C. A. Stott, printing and furnishing 10,000 letterheads | 43.75 | 8 |
| Aug. 2 | The Postmaster, mailing May 15, 1920 number | 25.00 | 9 |
| Aug. 10 | Janet K. Smith, 200 two-cent, 200 one-cent stamps | 6.00 | 11 |
| Aug. 10 | American Chemical Society, ½-page ad in *J. Ind. Eng. Chem.* | 105.98 | 12 |
| Aug. 11 | Macon Dray & Co., refund for excess payment on subscription | 1.20 | 13 |
| Aug. 15 | A. J. Holden, refund on journals which could not be furnished and $1 refund for excess payment on methods | 21.00 | 10 |
| Aug. 18 | Janet K. Smith, 1000 one-cent stamps | 10.00 | 14 |
| Aug. 21 | Proctor & Gamble Co., refund for excess payment on subscription | 1.43 | 16 |
| Aug. 30 | J. H. Gill, refund for excess payment on subscription | 1.00 | 17 |
| Aug. 31 | Jose L. Suarey, refund for excess payment on Book of Methods | .50 | 18 |
| Aug. 31 | A. Helsall, refund for excess payment on Book of Methods | .50 | 19 |
| Sept. 1 | Industrial Printing Co., on account | 500.00 | 20 |
| Sept. 3 | Peter McVeigh, refund for excess payment on Book of Methods | .50 | 21 |
| Sept. 10 | Industrial Printing Co., miscellaneous items | 87.78 | 22 |
| Sept. 16 | Industrial Printing Co., on account | 500.00 | 23 |

| | | | | |
|---|---|---|---|---|
| Sept. 18 | Industrial Printing Co., on account | | 500.00 | 24 |
| Sept. 18 | A. Zichtl & Co., binding Volume III, *This Journal* | | 2.75 | 25 |
| Sept. 20 | The Postmaster, box rent for quarter ending December 31, 1920 | | 2.00 | 26 |
| Sept. 22 | A. Poole Wilson, payment for copy of Volume III, No. 1 returned | | 1.40 | 27 |
| Oct. 2 | Joe Cohen, notarial services | | 1.25 | 28 |
| Oct. 2 | S. R. Curzon, refund for excess payment on Book of Methods. | | 1.16 | 29 |
| Oct. 4 | Industrial Printing Co., on account | | 500.00 | 30 |
| Oct. 5 | Industrial Printing Co., printing 2690 wrappers, 3000 labels | | 40.75 | 31 |
| Oct. 5 | P. J. Saxer, refund for excess payment on Book of Methods | | .50 | 32 |
| Oct. 5 | Henry Larouche, refund for excess payment on Book of Methods | | .50 | 33 |
| Oct. 12 | Postage | | 20.00 | 34 |
| Oct. 19 | W. G. Gaessler, for Volume I, No. 1, Volume II, No. 1, Part I | | 2.50 | 35 |
| Oct. 25 | Industrial Printing Co., on account | | 500.00 | 36 |
| Nov. 1 | Joe Cohen, notarial services | | .50 | 37 |
| Nov. 8 | Industrial Printing Co., on account | | 500.00 | 38 |
| Nov. 8 | Cash | | .13 | 26 |
| Nov. 12 | C. L. Alsberg, reimbursement Secretary-Treasurer account for checks drawn for Journal | | 762.48 | 40 |
| Nov. 12 | N. A. Parkinson, reimbursement freight charges on methods | | .75 | 41 |
| Nov. 6 | Bank balance | $2,586.79 | | |
| | Plus deposit of November 8 | 570.94 | | |
| | | $3,157.73 | | |
| | Less outstanding checks | 1,267.29 | | |
| | | | 1,890.44 | |
| | | | $9,481.35 | |

The undersigned committee has examined the above report and finds it correct.

Respectfully submitted,

A. J. PATTEN,
H. H. HANSON.

*Auditing Committee.*

Adopted.

## REPORT OF THE BOARD OF EDITORS.

By C. L. ALSBERG[1] (Bureau of Chemistry, Washington, D. C.), *Chairman.*

The circulation of the last number of *The Journal* that was issued, that is Volume III, Number 4, was 720 copies. Including the additional subscriptions received up to a few days ago, the circulation will be about 850 for the first number of Volume IV. Of course, at the present time it is impossible to say how many renewals there will be. In the past over two-thirds of the subscriptions have been renewed. The reason it is not possible at this time to make a definite statement of the total circulation of Volume III is that the majority of the subscriptions do

[1] Present address, Food Research Institute, Stanford University, Calif.

not begin with the first number of the volume but are scattered all through the volume. It is estimated that at the present rate of receiving new subscribers the subscription list should be at least 850 or 900 within the next few months but, of course, no definite estimate can be given. No serious effort has been made since last March by the management of *The Journal* to increase the subscription list because the work on the methods took up all the available time of those who otherwise would have been engaged on *The Journal.* Without any effort whatever to increase the circulation of *The Journal,* in the neighborhood of ten or a dozen new subscriptions a month have been received. Since the first of October some circularizing has been done, the work on the methods having been disposed of, and in the neighborhood of fifty subscriptions a month have been received. How long that will continue it is impossible to say. Should this rate of increase continue during the year, the circulation of *The Journal* would reach 1200, which would, in all probability, carry the cost of printing. At the present time, with a subscription list between seven and eight hundred, *The Journal* has a deficit.

It is also impossible to calculate from the receipts for *The Journal* this year exactly what the deficit is on each volume. The reason for this is that it has not been possible as yet to adjust our dispute with the former printers. Unfortunately, it was impossible to secure from the printer the list of original subscribers and it became necessary to build up a new list and begin all over again. In spite of this handicap, the list of subscribers is now within a couple of hundred of what it was when the publication of *The Journal* was suspended in May, 1917. The income, however, has not been proportionate because, of course, the association was under obligation to furnish the remainder of Volume III to such old subscribers as could be reached who had subscribed for it but had not received it, the money being tied up in the hands of the former printer who, under the contract, handled the business affairs of *The Journal.* To sum up, the total receipts for *The Journal* have been: In the form of subscriptions, $1733.17; in the form of advertising, $270; making a total of approximately $2000. The cost of printing *The Journal* has been such that this leaves a deficit of about $3200 on Volume III. It is not possible at the present time to estimate exactly what the prospects of income for Volume IV will be. There is on hand at present from subscriptions and advertising for Volume IV $2600, which, of course, will not meet the cost of production. If it is assumed that there is to be no material increase in the cost, and the number of subscriptions is placed somewhere in the neighborhood of 750, which corresponds to about the present circulation, and if it is assumed that there will be no reduction in the cost of printing, both of which estimates are ultraconservative since there has been a drop in the cost of

paper and printing and from thirty to fifty subscriptions a month are being received at the present time, then there is a possibility of a deficit slightly in excess of $3000 for Volume IV of *The Journal*. If, however, the cost of printing goes down somewhat, and if subscriptions continue to come in at the present rate, the deficit will be very much less. That may look like a serious situation, but in reality it is not.

It is impossible to submit a complete financial statement on the Book of Methods for the reason that the bills for printing and distribution have not been received. Only an estimate has been received as to the probable cost of producing the book and this estimate is in the neighborhood of $4000 and $4500. It can not be estimated exactly until all the bills are received. Up to the tenth of November, 1710 subscriptions to the Book of Methods had been received. On that basis, if everybody pays his bill, the money in sight for the Book of Methods, if not another copy were sold, is $8875.45, which should leave a surplus on the Book of Methods of something in the neighborhood of $4000. It may be as low as $3000; it may be as high as $4500. It is not possible at this time to estimate it. If not another copy were sold and only the money received in payment for the copies of the Book of Methods that have now been sold were received, the deficit on *The Journal* would be more than wiped out. This $8800 on the Book of Methods is not all that will be received because, although no circularizing to solicit subscriptions to the Book of Methods has been done, since the first of September an average of 39 subscriptions per week has been received for the Book of Methods. There is every prospect that from 25 to 50 a week will come along for several months at least, and perhaps for a year. The deficit on *The Journal* is due to the fact that a new subcription list had to be built up and the cost of printing has practically doubled since 1915. In connection with the building up of the subscription list, advertisements were inserted in the leading chemical journals announcing the resumption of publication of *The Journal*, and asking those who were subscribers and had paid for something that they had not received, to notify the association. It is unquestionably a fact that those advertisements were overlooked by a good many people, but they were the only means of reaching the former list of subscribers. There are several gentlemen in attendance at this meeting who did not see them, and who have asked about their old subscriptions. Any one who may have been a subscriber in the past and who has not received all his copies, is urged to notify the editorial office of that fact. In that event he is entitled to receive the remainder of the copies for which he has paid, and if any one knows of any of his colleagues of whom the same is true, please ask them to communicate with the editor's office. In short, the situation is this: *The Journal* at the present time probably is running a deficit of something around $1500, possibly $2000

a year. If several hundred former subscribers can be reached and renew their subscriptions, and the subscriptions are coming in at a rate which makes that probable, the deficit will be correspondingly less. About 1200 subscribers must be secured to break even. In other words, about 400 more subscriptions are needed. The methods are showing a profit which, at the present time, wipes out the deficit on *The Journal.* How long this will continue will depend, in the first place, upon how the subscription list to *The Journal* increases, and, in the second place, upon how continuous the demand remains for the Book of Methods.

Up to the present time exactly $7619.27 has been received for *The Journal* and for the Book of Methods, and there is $1890.44 in bank. That does not mean anything because it is impossible to estimate exactly what the cost of printing the Book of Methods will be because the bills are still outstanding. Part of the cost of producing the methods has been paid, but not all.

Number 1 of Volume IV is ready to go into the mail; Number 2 of Volume IV will follow within a month, and it is hoped that by spring the proceedings will be caught up completely. There is not any doubt that this will favorably affect the subscription list to *The Journal* because there are a certain number of subscribers who are interested only in the current proceedings and are not subscribing as long as back proceedings are being published. By spring it is hoped that *The Journal* will be in a position to print not merely proceedings, but such original communications coming into the field of work of this association as the Board of Editors may deem wise to furnish a place in *The Journal.*

It was moved, seconded and adopted that the report be received, that the thanks of the association be tendered to the Editor in Chief, and his able assistant, Miss N. A. Parkinson, and to the Board of Editors for the excellent and efficient service they rendered in connection with the publication of *The Journal* and the Book of Methods.

Considerable discussion followed the presentation of the Report of the Board of Editors. The question of publishing separates containing individual chapters from the Book of Methods was taken up in detail and a motion made that the Board of Editors be empowered to print the chapter on fertilizers as cheaply as possible for sale to students. Substitute motions were made later empowering the Board of Editors to print a students' edition of the entire Book of Methods or such part thereof as they deemed wise, on cheaper paper than the regular edition, which could be sold at a very much lower price. These motions were withdrawn, however, and a motion was made, seconded and carried that this matter be held over for one year at least.

# REPORT OF COMMITTEE ON RECOMMENDATIONS OF REFEREES.

By B. B. Ross (Alabama Polytechnic Institute, Auburn, Ala.), *Chairman.*

Your committee has no formal report to present other than that embodied in the several reports submitted by the chairmen of Subcommittees A, B and C, but deems it advisable to offer some suggestions relative to the desirabilty of securing earlier reports from the referees and associate referees. In this connection, the committee realizes that many of the collaborators must report more promptly to the referees, if a speeding up of the reports of the referees is to be attained. It is also desirable, if possible, to secure for the consideration of the several subcommittees, complete reports from the referees, or such abstracts as will set forth definitely the reasons upon which the recommendations of the referees are based. Many of the referees supply advance copies of their full reports, but others, owing possibly to delay in receipt of reports from collaborating chemists, or to the pressure of routine work, send in abstracts of their reports and, in some cases, these abstracts fail to give in sufficient detail the reasons for suggested changes or modifications in the methods. In some instances reports of collaborative work are not submitted.

The committee desires the referees and associate referees to supply each member of the appropriate subcommittee with copies of their reports and recommendations sufficiently well in advance of the meeting to permit the individual members of the committee to study and consider the report thoroughly prior to the date of the meeting of the association. In this way, the members of the various subcommittees will have an opportunity to become well posted on the several subjects to be considered by their committee and each committee will thus be better prepared to act promptly and intelligently upon reaching the meeting.

The committee realizes the difficulties which have confronted referees and collaborating chemists during the period of transition from a wartime status to normal working conditions, and hence does not make the above suggestions in a spirit of fault finding. It is hoped, however, that all members will appreciate the importance of pursuing the course outlined.

Adopted.

# REPORT OF SUBCOMMITTEE A ON RECOMMENDATIONS OF REFEREES.

By B. B. Ross (Alabama Polytechnic Institute, Auburn, Ala.), *Chairman.*

[Fertilizers (borax in fertilizers, preparation of ammonium citrate, precipitated phosphates, nitrogen, potash), potash availability, inorganic plant constitutents (sulfur and phosphorus in the seeds of plants, calcium and magnesium in the ash of seed), water, tanning materials and leather, insecticides and fungicides, and soils (sulfur in soils).]

## FERTILIZERS.

### BORAX IN FERTILIZERS.

It is recommended—

(1) That the Ross-Deemer method for the determination of borax in fertilizer materials and mixed fertilizers, which reads as follows, be adopted as a tentative method:

### ROSS-DEEMER METHOD FOR THE DETERMINATION OF BORIC ACID.

#### REAGENTS.

(a) *Barium chloride solution.*—(10%).

(b) *Barium hydroxide.*—Powdered.

(c) *Standard boric acid solution.*—(0.1N).

(d) *Standard sodium hydroxide solution.*—Prepare this solution free from carbonates by first making a saturated solution in order that any sodium carbonate present will be precipitated when the solution is allowed to stand in a vessel from which the carbon dioxide of the air is excluded. Filter through a hard filter that has been soaked in alcohol; dilute a portion to about 0.1N and accurately determine the strength of the solution by titration, as described under the determination of mineral salts, against the standard boric acid.

(e) *Hydrochloric acid solution.*—(1) About 0.1N and (2) about 0.5N.

(f) *Neutral mannite (mannitol).*

(g) *Methyl red solution.*—Dissolve 0.1 gram of methyl red in 100 cc. of a hot 50% solution of alcohol and water, and filter.

(h) *Phenolphthalein solution.*—Dissolve 1 gram of phenolphthalein in 100 cc. of alcohol.

#### DETERMINATION.

(a) *Mineral salts.*—Dissolve 5–10 grams of the sample in 50–75 cc. of hot water, decompose carbonates, if present, with a slight excess of hydrochloric acid; heat to boiling and add sufficient barium chloride to precipitate the sulfates, using about 10 cc. in excess; next add in small amounts sufficient powdered barium hydroxide to make the solution alkaline, avoiding a large excess; boil for about 5 minutes, or until any ammonia present has been expelled; filter and wash into a 300 cc. flask, make acid with hydrochloric acid, using an excess equivalent to a few cc. of 0.1N solution; boil for 15 minutes to expel carbon dioxide, cool by placing the flask in cold water and bring to neutrality by first adding 4–5 drops of methyl red and then standard sodium hydroxide solution until the color of the solution changes from pink to yellow. If the neutral point has been exceeded, or if there is any doubt as to this, restore the pink color by adding a few drops of approximately 0.1N hydrochloric acid and change the color to yellow again with the minimum amount of the standard sodium hydroxide solution.

Add 1–2 grams of neutral mannite and a few tenths of a cc. of phenolphthalein solution, note the buret reading, and again titrate the solution with the standard sodium hydroxide solution until a pink color develops. Add a little more mannite and if the pink color disappears continue the addition of the standard alkali until a pink color again appears. Repeat until the addition of mannite has no further action on the end point. If the content of boric acid in the solution titrated is low, one addition of mannite is usually sufficient. From the volume of the standard alkali required in the titration after the addition of the mannite, corrected for the volume required when running a blank, calculate the quantity of borax in the sample, 1 cc. of a 0.1N sodium hydroxide solution being equivalent to 0.0062 gram of boric acid, or to 0.00505 gram of anhydrous borax.

When an acid solution of the sample to be analyzed gives no precipitate upon the addition of a solution of calcium chloride and sufficient ammonia to give an alkaline reaction, phosphates and iron and aluminium salts are absent and that portion of the determination which involves treatment with barium chloride and barium hydroxide for the removal of these constitutents may then be omitted.

(b) *Mixed fertilizers and organic compounds.*—Weigh 5 grams of the sample into a 250 cc. beaker, add 50 cc. of hot water, cover with a watch glass, digest for 15–20 minutes on the water bath, filter and wash into another beaker of the same capacity. Heat the filtrate to boiling and add 15 cc. of barium chloride solution followed without undue loss of time by sufficient powdered barium hydroxide to give an alkaline reaction as indicated by phenolphthalein, boil for about 5 minutes, gently to prevent frothing over, filter and wash. Or, if preferred, make up to the mark in a graduated flask and take an aliquot portion. Evaporate the filtrate or aliquot portion to dryness in a platinum or porcelain dish and ignite the residue, preferably in a muffle furnace, at a temperature just below redness until organic matter is completely carbonized. Treat the ignited residue with hot water, make slightly acid with hydrochloric acid, heat nearly to boiling, make alkaline again with a slight excess of barium hydroxide and filter into a 300 cc. flask. Acidify with hydrochloric acid, using an excess equivalent to a few cc. of a 0.1N solution, boil to expel carbon dioxide and titrate as directed under the determination of mineral salts.

If the barium hydroxide has been added only in slight excess there is a tendency for the filtrate to become acid during evaporation with a possible loss of borax. It is therefore important that the solution be kept alkaline by repeated additions of barium hydroxide, if necessary, until the evaporation has been completed

If the filtrate from the barium chloride-barium hydroxide precipitate is titrated in this determination without first destroying soluble organic matter, the end points in the titration will usually be too indefinite to give accurate results. The purpose in evaporating the filtrate and igniting the residue is therefore to get rid of soluble organic constituents which interfere with the titration. When the sample contains a relatively high boric acid content, in excess of 0.5 per cent, a smaller sample may be taken and the quantity of organic matter present may then be too small to seriously interfere with the sharpness of the end points during the titration. When such is the case, boil the solution after the addition of the barium hydroxide until any ammonia present has been expelled, omit evaporating the filtrate from the barium chloride-barium hydroxide precipitate; add to the filtrate an excess of hydrochloric acid equivalent to a few cc. of a 0.1N solution, boil to expel carbon dioxide and titrate as directed under the determination of mineral salts.

Approved.

(2) That further work be done on the comparison of the proposed tentative method with the distillation method of Bartlett.

Approved.

(3) That results of determinations by these methods be reported hereafter in terms of boric acid, together with the equivalent of anhydrous borax.

Approved.

PREPARATION OF AMMONIUM CITRATE SOLUTION.

It is recommended—

(1) That the proposed method be given further study, with collaboration, with a view to adoption in 1921.

Approved.

PRECIPITATED PHOSPHATES.

It is recommended—

(1) That the proposed method be further studied, with collaboration, during the ensuing year.

Approved.

NITROGEN.

It is recommended—

(1) That the study of the du Pont nitrometer be abandoned.

Approved.

(2) That the associate referee for 1921 be directed to study the DeVarda alloy method[1] as applied to the determination of nitric and nitrous acids in fertilizers.

Approved.

POTASH.

No report or recommendations.

POTASH AVAILABILITY.

No recommendations.

INORGANIC PLANT CONSTITUENTS.

SULFUR AND PHOSPHORUS IN THE SEEDS OF PLANTS.

It is recommended—

(1) That the associate referee consider the suggestion for further work on total sulfur and total phosphorus.

Approved.

(2) That the associate referee consider the construction of a bomb similar to but larger than that recommended by Parr for the determination of sulfur in coal.

Approved.

(3) That the associate referee consider the use of sodium peroxide as an oxidizing agent with a small amount of potassium chlorate as an accelerator.

Approved.

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[1] *Chem. Ztg.*, 1892, **16**: 1952; *J. Ind. Eng. Chem.*, 1919, **11**: 306; 1920, **12**: 352.

(4) That the associate referee consider the suggestion that care be exercised to insure the complete oxidation of sulfur and phosphorus before it is neutralized with acid.

Approved.

(5) That the referee consider carrying on this work with a view to making the sulfur and phosphorus determinations on the same charge.

Approved.

### CALCIUM AND MAGNESIUM IN THE ASH OF SEED.

It is recommended—

(1) That further work be done on calcium and magnesium in such material as the ash of seed, as recommended in 1919.

Approved.

(2) That some cooperative work be done on the colorimetric method for manganese.

Approved.

(3) That a method be devised for the determination of iron and aluminium in the filtrate from magnesium, as recommended in 1919.

Approved.

### WATER.

It is recommended—

(1) That work on bromine be continued during the ensuing year.

Approved.

(2) With regard to the recommendation of the referee as to the insertion in the methods of analysis of the statement given below relative to the method for bromine in the presence of chlorine and iodine, the committee recommends that this statement be published in the proceedings of the association for the information of any chemists having occasion to make such determinations, pending the final adoption of a method.

A volumetric method for the determination of bromine in the presence of chlorine and iodine has been published[1]. Cooperative work indicates that this is probably the best method for bromine which has been published, but the results obtained show that only about 95 per cent of the bromine present is recovered, when 80 mg. of bromine are contained in the portion of the sample used for analysis. The method is satisfactory in the absence of iodine as shown by the cooperative work on water in 1919.

Approved.

### TANNING MATERIALS AND LEATHER.

No recommendations.

### INSECTICIDES AND FUNGICIDES.

It is recommended—

(1) That the hot bromate method for the titration of the acid dis-

[1] *J. Ind. Eng. Chem.*, 1920, **12**: 358.

tillate in the official distillation method for the determination of total arsenic[1] be adopted as an official method. (First reading.)

Approved.

(2) That the bromate method for the determination of arsenious oxide in Paris green[1] be adopted as an official method. (First reading.)

Approved.

(3) That the bromate method for the determination of arsenious oxide in calcium arsenate[2] be adopted as an official method. (First reading.)

Approved.

(4) That the official distillation method[3] as applied to the determination of total arsenic in London purple be adopted as an official method. (First reading.)

Approved.

(5) That the zinc oxide sodium carbonate method[4] for the determination of total arsenic in London purple be adopted as an official method. (First reading.)

Approved.

(6) That the bromate method for the determination of arsenious oxide in zinc arsenite[1] be adopted as an official method. (First reading.)

Approved.

(7) That the official method for the determination of water-soluble arsenic in lead arsenate[5] be adopted as an official method for the determination of water-soluble arsenic in zinc arsenite.

Approved.

(8) That the tentative method for the determination of arsenious oxide in lead arsenate[6] be adopted as a tentative method for the determination of arsenious oxide in calcium arsenate.

Approved.

(9) That the method for calcium oxide[7] be amended by eliminating the words, "or ignite and weigh as oxide", and when so amended that it be adopted as a tentative method.

Approved.

(10) That the modified method for calcium oxide[8] be adopted as a tentative method.

Approved.

(11) That in the "General procedure for the analysis of a product containing arsenic, antimony, lead, copper, zinc, iron, calcium, mag-

[1] *J. Assoc. Official Agr. Chemists*, 1921, 5: 34.
[2] *Ibid.*, 36.
[3] *Assoc. Official Agr. Chemists, Methods*, 1920, 54.
[4] *J. Assoc. Official Agr. Chemists*, 1921, 4: 397.
[5] *Ibid.*, 5: 47.
[6] *Assoc. Official Agr. Chemists, Methods*, 1920, 59.
[7] *J. Assoc. Official Agr. Chemists*, 1921, 5: 37.
[8] *Ibid.*, 41.

nesium, etc."[1] the methods for lead oxide and copper be adopted as official methods and the method for zinc oxide be adopted as a tentative method.

Approved.

(12) That the mercury-thiocyanate method for zinc oxide in zinc arsenite[2] be adopted as a tentative method.

Approved.

(13) That the official method for the determination of water-soluble arsenic in lead arsenate[2] be adopted, under suspension of the rules, as official for the determination of water-soluble arsenic in calcium arsenate. This method involves the same principle and method of procedure as are embodied in existing official methods for other materials.

Approved under suspension of the rules.

(14) That the official distillation method[3] be adopted, under suspension of the rules, as an official method for the determination of total arsenic in magnesium arsenate. This method involves the same principle and method of procedure as are employed in existing official methods for other materials.

Approved under suspension of the rules.

(15) That a study be made of methods for the determination of arsenious oxide, water-soluble arsenic and magnesium in magnesium arsenate.

Approved.

## SOILS.

### SULFUR IN SOILS.

It is recommended—

(1) That work be continued during the ensuing year in an effort to perfect the tentative method or some other procedure which will insure the complete recovery of total soil sulfur.

Approved.

[1] *J. Assoc. Official Agr. Chemists*, 1921, 5: 42.
[2] *Ibid.*, 47.
[3] *Assoc. Official Agr. Chemists, Methods*, 1920, 54.

# REPORT OF SUBCOMMITTEE B ON RECOMMENDATIONS OF REFEREES[1].

By H. C. LYTHGOE (State Department of Health, Boston, Mass.), *Chairman.*

[Foods and feeding stuffs (crude fiber, stock feed adulteration, water), saccharine products (sugar, honey, maple products, maltose products, sugar-house products), dairy products (moisture in cheese), testing chemical reagents, drugs (alkaloids, arsenicals, synthetic drugs, alkaloids of opium, medicinal plants, enzymes, sandalwood oil, balsam and gum resins.)]

## FOODS AND FEEDING STUFFS.

It is recommended that attention be given to the two following recommendations from 1917 and 1919 which have not been acted upon:

(1) That a further study be made of sulfur dioxide in bleached grain.

(2) That the method for determining the acidity of corn, as described by Black and Alsberg[2], be considered by the referee next year with a view to its adoption as an official method, and that the method be studied to determine whether changes are necessary to make it applicable to grains other than corn.

Your committee suggests that these two recommendations be referred to the referee on foods and feeding stuffs.

Approved.

It is further recommended—

(3) That the study of methods for the detection of ground bran in shorts, as outlined in papers by J. B. Reed[3] and D. B. Bisbee[4] be further studied during the coming year.

Approved.

## CRUDE FIBER.

It is recommended—

(1) That the method for crude fiber be further studied.

Approved.

A motion was made by G. S. Fraps that the words "or asbestos" be inserted after the word "linen" in the official method for crude fiber[5], lines 8 and 13; and that the deletion of the first and second sentences in the method for the determination of ether extract[6] be considered.

Your committee recommends that these questions be referred to the referee on foods and feeding stuffs and the associate referee on crude fiber respectively with directions to report at the next meeting.

Approved.

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[1] Presented by E. M. Bailey.
[2] U. S. Bur. Plant Ind. Bull. 199: (1910).
[3] *J. Assoc. Official Agr. Chemists*, 1921, 5: 70.
[4] *Ibid.*, 74.
[5] *Assoc. Official Agr. Chemists, Methods*, 1920, 98, 66.
[6] *Ibid.*, 72, 10.

### STOCK FEED ADULTERATION.

It is recommended—

(1) That the method recommended by the associate referee on stock feed adulteration[1] be further considered by the associate referee next year with a view to its adoption as a tentative method.

Approved.

### WATER.

Attention is called to the two following recommendations from 1919 which have not yet been acted upon:

(1) That the associate referee study the existing official general methods for water in foods and feeding stuffs with a view to rewording and fixing rigidly the conditions of temperature, pressure and other factors.

(2) That a definite method applicable to the determination of water in dried fruits be designed and submitted to the association.

Your committee recommends that these two recommendations be referred to the referee on foods and feeding stuffs for consideration next year.

Approved.

## SACCHARINE PRODUCTS.

### SUGAR.

Attention is called to the following 1916 and 1917 recommendations:

(1) That the modifications proposed in 1915 for determining sucrose by acid and invertase inversion be further studied.

(2) That the work upon determining small amounts of reducing sugars in the presence of sucrose be continued.

The committee suggests that these recommendations be referred to the referee on saccharine products.

Approved.

### HONEY.

No report or recommendations.

### MAPLE PRODUCTS.

No report or recommendations.

### MALTOSE PRODUCTS.

No report or recommendations.

### SUGAR-HOUSE PRODUCTS.

It is recommended that the following 1919 recommendations be continued—

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[1] *J. Assoc. Official Agr. Chemists*, 1921, 5: 77.

(1) That a study be made of the influence of different and known temperatures of incineration on the results of ash determinations in cane sirups and molasses, carrying out the incineration in both platinum and silica dishes for comparison.

Approved.

(2) That a large number of samples of different grades of cane sirups and molasses be used for comparing ash determinations by the sulfate and direct methods, to determine, if possible, the proper correction factor to be applied to the sulfated ash.

Approved.

DAIRY PRODUCTS.

It is recommended—

(1) That a further study be made of the alkaline acid modification of the Roese-Gottlieb method as applied to dried milk products of various fat content.

Approved.

(2) That a further study be made of the Roese-Gottlieb neutral extraction method as applied to malted milk.

Approved.

(3) That a further study be made of a direct ether extraction method as applied to malted milk.

Approved.

Attention is called to a recommendation in 1917 that the Schmidt-Bondzynski method for the determination of fat in cheese be adopted as official. (First reading.) In 1919 it was recommended that this method be further studied. Your committee would, therefore, recommend—

(4) That this method be reported upon by the referee next year.

Approved.

(5) That collaborative work be done upon the cryoscopic examination of milk with a view to making the method official.

Approved.

MOISTURE IN CHEESE.

No report or recommendations.

TESTING CHEMICAL REAGENTS.

It is recommended—

(1) That this association declare itself in favor of cooperating with the Committee on Guaranteed Reagents and Standard Apparatus of the American Chemical Society in the collection of data in regard to the quality of reagents on the market.

Approved.

(2) That the secretary of this association be instructed to transmit a statement of this action to the proper official of each institution rep-

resented in the membership of the association and request that the purchasing agent or some other official of the institution send him a carbon copy of each letter written to a manufacturer or dealer calling attention to a specific instance of delivery of an unsatisfactory reagent.

Approved.

DRUGS.

It is recommended—

(1) That a representative of this association be appointed to collaborate with the revision committee of the United States Pharmacopœia and report progress at the next annual meeting of the association.

Approved.

(2) That an associate referee be appointed to study the methods of examination of acetylsalicylic acid reported by the referee[1], or any method or methods that may be available elsewhere, for the purpose of selecting or developing the most satisfactory method or methods of analysis.

Approved.

(3) That an associate referee be appointed to study the methods for the examination of phenolphthalein reported by the referee[2], or any method or methods that may be available elsewhere, for the purpose of selecting or developing a satisfactory method or methods of analysis.

Approved.

(4) That an associate referee be appointed to study the methods for the examination of camphor and camphor preparations reported upon by the referee[2], or any method or methods that may be available elsewhere, for the purpose of selecting or developing a satisfactory method or methods of analysis.

Approved.

(5) That an associate referee be appointed to study the methods for the examination of mercurous chloride, mercuric chloride, and mercuric iodide reported upon by the referee[3], or any method or methods that may be available elsewhere, for the purpose of selecting or developing satisfactory methods of analysis.

Approved.

(6) That an associate referee be appointed to study the methods for the detection of mineral oils in turpentine reported upon by the referee[4], or any method or methods that may be elsewhere available, for the purpose of selecting or developing additional methods for the detection of mineral oils in turpentine.

Approved.

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[1] *J. Assoc. Official Agr. Chemists*, 1921, 5: 141.
[2] *Ibid.*, 143.
[3] *Ibid.*, 145.
[4] *Ibid.*, 148.

(7) That an associate referee be appointed to study the methods of examination of papain reported by the referee[1], or any method or methods that may be elsewhere available, for the purpose of selecting or developing satisfactory methods of analysis.

Approved.

ALKALOIDS.

It is recommended—

(1) That the methods submitted for the separation of quinine and strychnine be further studied by collaborators.

Approved.

(2) That the method for the assay of physostigma and its preparations be studied by collaborators.

Approved.

(3) That the method for the assay of fluidextract of hyoscyamus be studied by collaborators.

Approved.

(4) That the comparative study of volumetric and gravimetric methods for the assay of ipecac be subjected to collaborative investigation.

Approved.

ARSENICALS.

It is recommended—

(1) That the methods reported by the associate referee[2] or any other methods that may be otherwise available be studied with a view to selecting a satisfactory method or methods of analysis.

Approved.

SYNTHETIC DRUGS.

It is recommended—

That the method for the valuation of hexamethylenetetramine tablets presented to the association in 1916 together with results of collaborative work[3] be adopted as tentative.

Approved.

(2) That the method of W. O. Emery for the estimation of monobromated camphor in migraine tablets[4] be studied cooperatively; and that the method of E. O. Eaton be studied further.

Approved.

(3) That the method of S. Palkin for the determination of phenolphthalein[5] be studied cooperatively and that further study be made of other methods.

Approved.

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[1] *J. Assoc. Official Agr. Chemists*, 1921, 5: 146.
[2] *Ibid.*, 149.
[3] *Ibid.*, 1920, 3: 374.
[4] *J. Ind. Eng. Chem.*, 1919, 11: 756.
[5] *Ibid.*, 1920, 12: 766.

(4) That the method submitted for the examination of procaine[1] be studied by collaborators during the coming year.

Approved.

ALKALOIDS OF OPIUM.

It is recommended—

(1) That the methods submitted by the associate referee for the examination of morphine, codeine, and heroine[2] be studied by collaborators.

Approved.

MEDICINAL PLANTS.

It is recommended—

(1) That further work be done on the value of weights of unit volumes or the specific weight of crude drugs and spices.

Approved.

(2) That the subject of sublimation for analysis of plant products, etc., be further studied.

Approved.

(3) That the methods for the macroscopic and microscopic identification of *Digitalis thapsi* (Spanish digitalis), a recent substitute for *Digitalis purpurea*, and *Hyoscyamus muticus* (Egyptian henbane), a substitute for *Hyoscyamus niger*, be studied by collaborators.

Approved.

(4) That the method for the detection of the presence of santonin in wormseed (*Artemisia cina*), and subsequent isolation, be studied by collaborators.

Approved.

(5) That the method for the use of pollen grains as a means of identification and differentiation of plants and plant products be further studied.

Approved.

(6) That further information be collected concerning adulterants and substitutes of crude drugs and spices.

Approved.

ENZYMES.

No report or recommendations.

SANDALWOOD OIL.

It is recommended—

(1) That the methods submitted by C. W. Harrison at the 1919 meeting for the determination of the acetyl value of sandalwood oil[3] be further studied by collaborators.

Approved.

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[1] *J. Assoc. Official Agr. Chemists*, 1921, 5: 164.
[2] *Ibid.*, 150.
[3] *Ibid.*, 4: 425.

BALSAM AND GUM RESINS.

It is recommended that the following 1919 recommendation be referred to the referee on drugs for next year.

(1) That further collaborative work be done upon the method submitted for the determination of crude fiber in gum karaya.

Approved.

## REPORT OF SUBCOMMITTEE C ON RECOMMENDATIONS OF REFEREES.

By R. E. DOOLITTLE (Transportation Building, Chicago, Ill.), *Chairman.*

[Food preservatives (saccharin), coloring matters in foods, metals in foods, fruits and fruit products (pectin in fruit products), canned foods (physical methods of examination, tomato products), cereal foods, wines, distilled liquors, beers (limits of accuracy in the determination of small amounts of alcohol), methods of analysis of near beers, soft drinks (bottlers' products), vinegars, flavoring extracts, meat and meat products (separation of meat proteins, decomposition of meat products, gelatin), fats and oils, eggs and egg products, spices, cacao products (determination of shells, examination of cacao butter), coffee, tea, baking powder.]

FOOD PRESERVATIVES (SACCHARIN).

No report or recommendations received from the referee. Your committee, however, recommends that the following recommendations adopted at the 1917 and 1919 meetings, be continued.

(1) That further work be done on the method for the determination of saccharin in the presence of mustard oil[1].

Approved.

(2) That other methods not dependent upon the sulfur component of saccharin be investigated.

Approved.

(3) That further work be done upon the determination of saccharin in baked flour preparations.

Approved.

COLORING MATTERS IN FOODS.

It is recommended—

(1) That the methods submitted by the referee[2] for the determination of moisture, total matter insoluble in water, inorganic or nonvolatile matter insoluble in water, total matter soluble in water, matter insoluble in carbon tetrachloride, sodium chloride, sodium sulfate, sulfated ash, heavy metals, calcium, arsenic by direct precipitation, arsenic after

[1] *J. Assoc. Official Agr. Chemists*, 1920, 3: 505.
[2] *Ibid.*, 1921, 5: 196.

treatment with nitric acid, total arsenic, sulfur, nitrogen, total halogens, total iodine, sodium iodide, ether extractives, dye by titration with titanium trichloride, dye by titration with potassium permanganate, dye by colorimetric comparison, lower sulfonated dyes, melting point, Martius Yellow and Naphthol Yellow S, boiling point of Cumidine from Ponceau 3R, Orange II and Orange I, Iodeosine G in Erythrosine, and isomeric and similar dyes in Amaranth for the examination of commercial food colors be adopted as tentative and that they be subjected to collaborative study during the coming year with a view to the adoption by the association as official methods for the examination of commercial food colors.

Approved.

(2) That further study be made of methods applicable to the separation of the oil-soluble coal tar food colors from fats and oils.

Approved.

(3) That work on the common natural colors be continued.

Approved.

(4) That the referee give consideration to the tentative methods on coloring matters in foods, Chapter X[1], during the coming year for the purpose of recommending such as are considered suitable for adoption as official methods.

Approved.

METALS IN FOODS.

It is recommended—

(1) That the tentative methods for copper and zinc[2], together with any other apparently desirable methods, be studied by collaborative work during the coming year.

Approved.

(2) That the Gutzeit method and apparatus for the determination of arsenic, described by H. V. Farr but as yet unpublished, be studied by collaborative work in comparison with the present Gutzeit method.

Approved.

(3) That the Penniman method for tin[3] be studied further with a view to revision or radical modification and that collaborative study be made of any promising procedure that may be developed.

Approved.

(4) That metals for which no methods had been suggested be studied in the order of their toxicity, the likelihood of their occurrence in foods being given first consideration.

Approved.

(5) That the attention of the referee be called to the action by the

[1] *Assoc. Official Agr. Chemists, Methods*, 1920, 131.
[2] *Ibid.*, 151.
[3] *J. Assoc. Official Agr. Chemists*, 1920, 4: 172.

association at the 1919 meeting, making the gravimetric method and the volumetric method for the determination of tin, official methods. (First action.)

Approved.

FRUITS AND FRUIT PRODUCTS.

It is recommended—

(1) That the methods submitted by F. B. Power[1] for the detection of methyl anthranilate in fruit juices be referred to the referee for collaborative study during the coming year.

Approved.

PECTIN IN FRUIT AND FRUIT PRODUCTS.

It is recommended—

(1) That further investigation be made of methods for the detection of pectin in jellies, jams and similar fruit products.

Approved.

CANNED FOODS.

It is recommended—

(1) That the investigation of methods for the detection of spoilage and for distinguishing conditions which are likely to lead to spoilage be continued.

Approved.

PHYSICAL METHODS OF EXAMINATION.

No report or recommendations.

TOMATO PRODUCTS.

No report or recommendations.

CEREAL FOODS.

It is recommended—

(1) That the work on the determination of moisture, gluten, soluble carbohydrates, cold water extract, chlorine and ash be continued.

Approved.

(2) That the referee study methods for the determination of fat in baked cereal products.

Approved.

WINES.

No report or recommendations.

DISTILLED LIQUORS.

No report or recommendations.

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[1] *J. Am. Chem. Soc.*, 1921, **43**: 377.

### BEERS (LIMITS OF ACCURACY IN THE DETERMINATION OF SMALL AMOUNTS OF ALCOHOL).

It is recommended—

(1) That the study of the method for the determination of alcohol to determine limits of accuracy be continued.

Approved.

### NEAR BEERS.

No report or recommendations.

### SOFT DRINKS (BOTTLERS' PRODUCTS).

It is recommended—

(1) That the study of methods for the examination of this class of products be continued.

Approved.

### VINEGARS.

It is recommended—

(1) That the methods for the determination of glycerol, solids, and fixed acids be studied during the coming year.

Approved.

### FLAVORING EXTRACTS.

It is recommended—

(1) That a study of methods for the analysis of imitation vanilla preparations containing large quantities of coumarin and vanillin be made.

Approved.

(2) That the method suggested by Penniman and Randall for the determination of oil in lemon and orange extracts[1] be studied in connection with the official method.

Approved.

(3) That a study of methods for the examination of non-alcoholic extracts be made.

Approved.

(4) That the method adopted at the 1919 meeting of the association as an official alternative method (first action) for the determination of alcohol in orange and lemon extracts consisting only of alcohol, oil and water[2] be subjected to collaborative study with a view to recommendation for final action at the 1921 meeting.

Approved.

### MEAT AND MEAT PRODUCTS.

It is recommended—

(1) That the method for the determination of sugar in meat and

[1] *J. Ind. Eng. Chem.*, 1914, 6: 926.
[2] *Ibid.*, 1909, 1: 84.

meat products[1], adopted at the 1919 meeting as tentative, be subjected to collaborative study during the coming year.

Approved.

SEPARATION OF MEAT PROTEINS.

It is recommended that the two following recommendations adopted at the 1919 meeting be continued.

(1) That further work be done on the Schlösing-Wagner method for the determination of nitrates, using beef extract, and other meat products.

Approved.

(2) That the associate referee attempt to determine the relative amounts of the dissociation products in water-soluble and water-insoluble meat proteins.

Approved.

(3) That the attention of the associate referee be called to the action taken by the association at the 1919 meeting whereby the ferrous chloride method for the determination of nitrates was changed to express the results in terms of sodium nitrate while no action was recommended by the associate referee in connection with the phenoldisulfonic acid method for the same determination.

Approved.

DECOMPOSITION OF MEAT PRODUCTS.

No report or recommendations.

GELATIN.

It is recommended—

(1) That the methods, submitted by the referee shortly after the adjournment of the 1919 meeting, for the determination of moisture, ash, total phosphorus, nitrogen, arsenic, copper, lead, zinc, polariscopic constants, and sulfur dioxide for the examination of gelatin be adopted as tentative methods, and that these methods be referred to the associate referee for collaborative study during the coming year.

Approved.

These methods are as follows:

*Moisture.*

(a) Proceed as directed under VII, 3[2].

(b) Dry in water oven at 100°C. for 6 hours. Cool in a vacuum desiccator admitting air dried by passing through concentrated sulfuric acid and weigh rapidly with dish covered.

(c) Dry in vacuum oven at 70°C. and cool in a vacuum desiccator admitting air as in (b).

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[1] *Assoc. Official Agr. Chemists, Methods*, 1920, 213.
[2] *Ibid.*, 71.

### *Ash.*

Ash at low redness preferably in a muffle as directed under VII, 4[1].

### *Total Phosphorus.*

Treat the ash obtained as above with 2–3 cc. of nitric acid (sp. gr. 1.42) and evaporate on the steam bath. Repeat the nitric acid treatment and take up in hot water containing a few drops of nitric acid and proceed as directed under I, 6[2].

### *Nitrogen.*

Proceed as directed under XX, 6[3].

### *Arsenic*[4].

Heat 20 grams of gelatin with 75 cc. of arsenic-free hydrochloric acid, 1 to 3, in a covered vessel until all insoluble matter has flocculated and the gelatin dissolved. Add an excess of bromine water (about 20 cc.), neutralize with ammonium hydroxide; add either about ½ cc. of 85% phosphoric acid or 2 grams of sodium phosphate ($Na_2HPO_4.12H_2O$), or crystallized sodium ammonium phosphate and allow to cool. Precipitate the arsenic acid along with the phosphoric acid by an excess of magnesia mixture (cf. I, 4, (c))[5]. The phosphoric acid or compound used should require about 20–25 cc. of the usual magnesia mixture for precipitation. After standing about an hour, wash the precipitate several times with dilute ammonium hydroxide, drain well and dissolve in dilute hydrochloric acid, 1 to 3, to 50 cc. volume in a graduated flask. Take a 25 cc. aliquot and determine the arsenic as directed under XI, 4[6]. Run a blank determination with the sample. Arsenic impurities, if present, are usually found in the phosphate added.

### *Copper.*

Hydrolize 50 grams of gelatin with 150 cc. of dilute hydrochloric acid, 1 to 3, as directed under arsenic, heating about 2 hours on the steam bath. To facilitate filtration and separation from zinc and iron later, use the phosphoric acid or compound and magnesia mixture as before. Precipitate with hydrogen sulfide in a slightly ammoniacal solution. Allow the precipitate to settle, filter and wash with 5% ammonium chloride solution saturated with hydrogen sulfide. Dissolve off the zinc and iron sulfides, magnesium phosphate, etc., in 75 cc. of dilute hydrochloric acid (4% HCl) saturated with hydrogen sulfide. Digest filter and copper sulfide with 4 cc. of concentrated sulfuric acid and sufficient nitric acid until the residue is perfectly colorless and fuming freely. Take up with water and determine copper by titrating with 0.01N sodium thiosulfate as directed under VII, 28[7].

### *Lead.*

If lead is present, it is shown as the sulfate mixed with some silica when the sulfuric acid residue is diluted with water in the above determination. Add an equal volume of alcohol and allow to stand several hours. Filter and wash with dilute alcohol. Evaporate the filtrate to remove alcohol and determine copper as directed under VII, 28[7].

Dissolve the lead sulfate from the filter with 10 cc. of hot 50% ammonium acetate solution alternated with hot water until the filtrate measures about 75 cc. Add pot-

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[1] *Assoc. Official Agr. Chemists, Methods*, 1920, 71.
[2] *Ibid.*, 2.
[3] *Ibid.*, 209.
[4] U. S. Bur. Chem. Circ. 102; *J. Soc. Chem. Ind.*, 1907, 26: 1115.
[5] *Assoc. Official Agr. Chemists, Methods*, 1920, 2.
[6] *Ibid.*, 148.
[7] *Ibid.*, 79.

assium dichromate solution to precipitate the lead as chromate, filter on a Gooch, dry at 125–150°C. and weigh. Calculate to metallic lead using the factor 0.641.

*Zinc.*

Determine the zinc in filtrate from copper determination as directed under **XI, 9**[1], or as directed below beginning with "Boil the filtrate, etc."

*Alternate Method for Copper and Zinc*[2].

Hydrolize 20–50 grams of gelatin with 100 cc. of dilute hydrochloric acid, 1 to 3, for 2 hours on the steam bath. Add 5 mg. of iron from 5 cc. of a standard solution of ferrous sulfate (4.9 grams of ferrous sulfate to a liter containing 10 cc. of sulfuric acid). Make solution faintly ammoniacal and saturate with hydrogen sulfide. Filter the sulfides and wash 2 or 3 times with a very dilute solution of colorless ammonium sulfide (saturate a solution of 1 cc. of concentrated ammonium hydroxide in 200 cc. of water). Dissolve the sulfides in 20 cc. of hot, dilute nitric acid, 1 to 1, and wash filter and insoluble matter with water. Add 10 cc. of dilute sulfuric acid, 1 to 3, and evaporate all the nitric acid. Cool and add 40 cc. of water. When the soluble salts are in solution filter off silica, washing filter thoroughly with water. Saturate the filtrate with hydrogen sulfide. Heat the solution 5 minutes on the steam bath. Filter the copper sulfide on a carefully prepared Gooch crucible and wash with hydrogen sulfide water. Dry and ignite to copper oxide.

Boil the filtrate to expel all hydrogen sulfide. Make the solution strongly ammoniacal and then acidify with 15 cc. of 50% formic acid. Filter off any insoluble matter such as alumina, etc., while hot, and then pass in a rapid stream of hydrogen sulfide for 10 minutes. Warm solution 15 minutes on the steam bath, remove and allow to stand for 30 minutes before filtration. Filter the zinc sulfide on a carefully prepared Gooch crucible with a very gentle suction, washing with 2% ammonium thiocyanate. Dry and ignite at the highest temperature of a Bunsen burner. Cool and weigh the zinc oxide.

*Polariscopic constants.*

Prepare a concentration of 3 grams per 100 cc. by soaking 3 grams of the sample in 40–50 cc. of cold water for about 15 minutes, heating to complete solution at about 50°C. and making to volume at 35°C. Polarize at 35°C. in a 2 dm. tube using the Ventszke scale.

Cool a portion of the gelatin solution rapidly to 10–15°C. and pour into cold, dry 1 dm. tubes before jelly has had time to form. Place the tube in a constant temperature bath at 15°C. and polarize after 18 hours to obtain equilibrium rotation at 15°C. Double the reading to place it on basis of 2 dm. tube.

In order to polarize cloudy samples, digest the original 100 cc. in a stoppered flask with roughly 10 cc. of lightly powdered magnesium carbonate for at least 1 hour at 35–40°C. and filter until clear through a folded filter, avoiding unnecessary evaporation.

The increase in levorotation (mutarotation) between 35° and 15° is an index of the jelly strength developed.

*Sulfur dioxide.*

Proceed as in the distillation method under **IX, 31**[3] or by the diffusion method as follows: Cool, in ice water, a vessel containing 100–150 cc. of water, 5 cc. of dilute hydrochloric acid, 1 to 3, 10 grams of sodium chloride and some filtered starch solution.

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[1] *Assoc. Official Agr. Chemists, Methods*, 1920, 151.
[2] *J. Ind. Eng. Chem.*, 1919, **11**: 323.
[3] *Assoc. Official Agr. Chemists, Methods*, 1920, 127.

Add a few drops of 0.01N iodine until a blue color is produced. Pour this mixture on 5 grams of powdered gelatin sample contained in a stoppered flask, replacing in the ice water. After remaining for 2 minutes with occasional mixing, add 0.01N iodine until the blue color is restored. Replace in the ice water for one minute, remove and titrate to the reappearance of the color. Repeat these operations until the color persists for 1 minute.

One cc. of 0.01N iodine is equivalent to 0.32 milligram of sulfur dioxide.

### FATS AND OILS.

It is recommended—

(1) That the Wijs method for the determination of iodine absorption number[1] be made official. (First reading.)

Approved

### EGGS AND EGG PRODUCTS.

It is recommended—

(1) That the method presented by the referee for the determination of zinc in dried egg products[2] be made a tentative method and that it be referred to the referee for collaborative study during the coming year.

Approved.

(2) That the W. G. McGeorge method for the determination of zinc in dried egg products[3] be studied during the coming year.

Approved.

(3) That the study of the methods for the determination of lecithin-phosphoric acid in dried eggs and alimentary pastes be continued.

Approved.

### SPICES.

It is recommended—

(1) That the studies of the distillation method for the determination of water in whole spices be continued.

Approved.

(2) That the method for the determination of volatile oil in mustard seed[4], which was adopted as an official method (first reading) at the 1919 meeting, be subjected to collaborative study during the coming year with a view to its presentation for final action, if possible, at the 1921 meeting.

Approved.

(3) That the paper, entitled "Salad Dressings and Their Analyses", by H. A. Lepper[5], be referred to the referee on spices for the study of the methods contained therein.

Approved.

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[1] *Assoc. Official Agr. Chemists, Methods*, 1920, 245.
[2] *J. Assoc. Official Agr. Chemists*, 1921, 5: 192.
[3] *Ibid.*, 194.
[4] *Assoc. Official Agr. Chemists, Methods*, 1920, 259.
[5] *J. Assoc. Official Agr. Chemists*, 1921, 5: 248.

(4) That the method for the detection of molds in drugs, foods and spices, by means of the chitin test[1] be adopted as a tentative method.

Approved.

## CACAO PRODUCTS.

### DETERMINATION OF SHELLS.

It is recommended—

(1) That the microscopic methods for the estimation of shells in cacao products be studied further.

Approved.

(2) That the chemical methods, particularly crude fiber, for the estimation of shells be studied in order to determine whether conclusions drawn from these methods may be correlated with those obtained by the microscopic examination.

Approved.

(3) That the paper, entitled "Cacao Products with Special Reference to Shell Content", by B. H. Silberberg[2], be referred to the referee on the determination of shells in cacao products for his information and study of methods.

Approved.

(4) That the association recommend to the Committee on Cooperation with Other Committees on Food Definitions a modification of the standards on cacao products[3]. Your committee does not approve this recommendation but suggests that the referee refer his data and in-information directly to the Secretary of the Joint Committee on Definitions and Standards.

Committee recommendation approved.

### CACAO BUTTER.

It is recommended—

(1) That further collaborative work be done on the critical temperature of dissolution method, especially to test the accuracy of the correction factor for acidity.

Approved.

(2) That the tests for tallow, hydrogenated oils, lard, paraffin, etc., be further studied.

Approved.

## COFFEE.

It is recommended—

(1) That the Power-Chesnut method for the determination of caffeine in coffee[4] be adopted as an official method. (First reading.)

Approved.

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[1] *J. Assoc. Official Agr. Chemists*, 1921, 5: 156.
[2] *Ibid*, 260.
[3] U. S. Dept. of Agr., Office of the Secretary, Circ. 136: (1919), 18.
[4] *J. Assoc. Official Agr. Chemists*, 1921, 5: 271.

(2) That the Fendler-Stüber method for the determination of caffeine in coffee be retained as a tentative method and be designated as a method to be used when quick results are desired.

Approved.

(3) That the Stahlschmidt method for the determination of caffeine in coffee be dropped from the official and tentative methods.

Approved.

(4) That the referee study methods for the determination of other constituents of coffees.

Approved.

(5) That the paper, entitled "Robusta Coffee", by A. Viehoever and H. A. Lepper[1], be referred to the referee for his information and study of methods.

Approved.

TEA.

It is recommended—

(1) That the modified Stahlschmidt method for the determination of caffeine in tea[2] be not made official this year.

Approved.

(2) That the Power-Chesnut method for the determination of caffeine in tea[3] be made official. (First reading.)

Approved.

(3) That the proposed method for the determination of caffeine[4] be studied collaboratively during the coming year.

Approved.

BAKING POWDERS.

It is recommended—

(1) That a further study be made of the Chittick method[5] for the determination of lead in baking powder with a view to establishing it as a tentative method.

Approved.

(2) That a study be made of the details of the electrolytic method for the determination of lead in baking powder with special reference to the acidity conditions during electrolysis.

Approved.

(3) That a study be made of methods for the determination of the neutralizing strength of baking acids.

Approved.

(4) That the method for the determination of fluorine in baking

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[1] *J. Assoc. Official Agr. Chemists*, 1921, 5: 274.
[2] *Assoc. Official Agr. Chemists, Methods*, 1920, 270.
[3] *J. Assoc. Official Agr. Chemists*, 1921, 5: 290.
[4] *Ibid.*, 291.
[5] *J. Assoc. Official Agr. Chemists*, 1920, 4: 218.

powder and phosphates adopted as a tentative method at the 1919 meeting[1] be subjected to collaborative study during the coming year.

Approved.

(5) That the paper, entitled "Determination of Total Carbon Dioxide in Baking Powder"[2], by C. S. Robinson, be referred to the referee on baking powder for study of the method contained therein.

Approved.

## REPORT OF THE COMMITTEE TO COOPERATE WITH OTHER COMMITTEES ON FOOD DEFINITIONS[3].

Your committee has no formal report to present. Because of the withdrawal from the committee of three of its members, E. F. Ladd, F. C. Blanck, and J. S. Abbott, no sessions of the committee have been held since the last meeting of this association. Work is progressing on a number of subjects, however, particularly canned foods, but has not reached such a stage that it can be reported at this time.

Respectfully submitted,

WILLIAM FREAR,
JULIUS HORTVET,
C. D. HOWARD.

*Committee to Cooperate with Other Committees on Food Definitions.*

President Lythgoe read a letter from H. E. Howe, Chairman of the Division of Research Extension of the National Research Council, inviting this association to appoint two members to serve on the Board of Trustees of the Crop Protection Institute. A motion was made, seconded and duly carried that this matter be referred to the incoming Executive Committee with power to act.

Adopted.

## REPORT OF COMMITTEE ON RESOLUTIONS[3].

Since the 1919 meeting of this association, word has come to your committee of the death of two valued members.

Albert F. Seeker, for some years Chief of the New York Food and Drug Inspection Station of the Bureau of Chemistry, died on August 19, 1919, from an attack of appendicitis. A skilful analyst and a chemist of unusually broad information in the domain of this science, Mr. Seeker performed service of conspicuous value to this association as referee on

[1] *J. Assoc. Official Agr. Chemists*, 1921, 4: 585.
[2] *Ibid.*, 5: 182.
[3] Presented by William Frear.

the subjects of spices, flavoring extracts and food preservatives, and, as a member of the Committee on Editing Methods of Analysis, bore a heavy share of that responsibility.

Your committee recommends the adoption of the following resolution:

*Resolved*, That in the death of Albert F. Seeker this association has lost a member who for years made contributions of very high value to its work, and its members, a friend who had won the esteem of all for his quiet manliness and his spirit of helpfulness.

*Resolved*, That the editor of *The Journal* be requested to print an appropriate notice of Mr. Seeker's work and death.

In January, 1920, after an illness of a few days, died Hugh A. Bryan, at that time Chief Chemist to Arbuckle and Company. Dr. Bryan, as chief chemist of the Sugar Laboratory of the Bureau of Chemistry, was for years an active member of this association. He won the high regard of the members for his geniality and his studious industry, and rendered invaluable service to the association as its referee on sugar and related products. An account of the life and service of Dr. Bryan has already appeared in this *Journal*[1].

Your committee recommends the adoption of the following resolution:

*Resolved*, That in the death of Dr. Hugh A. Bryan, this association has lost a highly prized associate member, from whom during his years of active membership it received signal aid in its work upon sugar chemistry, and from whom, as a fellow analyst in the prime of his power, his associates had confidently expected further scientific contributions of great value.

*Resolved*, That the secretary of the association be directed to send copies of these resolutions of appreciation to the families of the deceased members.

*Resolved*, That the Association of Official Agricultural Chemists hereby expresses to the Honorable Edwin T. Meredith, Secretary of Agriculture, its thanks for his address before this body, and particularly for his appreciation of the worth of the services of this association to the work of his department and to agricultural progress.

*Resolved*, That a vote of thanks be extended by the association to our President, Mr. Hermann C. Lythgoe, for the admirable manner in which he has conducted the proceedings of this convention.

*Resolved*, That this association extend a vote of appreciation and thanks to its secretary and to his faithful assistant, Miss N. A. Parkinson, for the able manner in which they have performed their work in connection with this convention, for their excellent services in the

[1] *J. Assoc. Official Agr. Chemists*, 1920, 3: iii.

handling and preparation of our reports for publication in *The Journal*, and also for their supervisory work in connection with the publication of the Official and Tentative Methods of Analysis.

*Resolved*, That this association extend its hearty thanks to the Board of Editors and to the Committee on Editing Methods of Analysis for the able and faithful manner in which they have performed the arduous duties imposed on them.

*Resolved*, That the Association of Official Agricultural Chemists extend its thanks to the management of the New Willard Hotel for the use of the ball room and for the other conveniences which have been granted to the association, and for the many courtesies which have been extended to our members.

*Resolved*, That the secretary be and is hereby directed to transmit to each of the respective persons named in these resolutions, a copy of the appropriate resolution.

Respectfully submitted,

WILLIAM FREAR,
JULIUS HORTVET,
E. W. MAGRUDER.
*Committee on Resolutions.*

The convention adjourned.

# PROCEEDINGS OF THE THIRTY-SEVENTH ANNUAL CONVENTION OF THE ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS, 1921.

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## OFFICERS, COMMITTEES, REFEREES, AND ASSOCIATE REFEREES OF THE ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS, FOR THE YEAR ENDING OCTOBER, 1922.

*Honorary President.*

H. W. WILEY, Woodward Building, Washington, D. C.

*President.*

F. P. VEITCH, Bureau of Chemistry, Washington, D. C.

*Vice-President.*

A. J. PATTEN, Agricultural Experiment Station, E. Lansing, Mich.

*Secretary-Treasurer.*

W. W. SKINNER, Bureau of Chemistry, Washington, D. C.

*Additional Members of the Executive Committee.*

H. D. HASKINS, Agricultural Experiment Station, Amherst, Mass.
R. E. DOOLITTLE, Transportation Building, Chicago, Ill.

PERMANENT COMMITTEES.

*Cooperation with Other Committees on Food Definitions.*

William Frear (State College, Pa.), *Chairman.*
Julius Hortvet, St. Paul, Minn.
C. D. Howard, Concord, N. H.

*Membership of Committee to Cooperate in Revision of the U. S. Pharmacopœia.*

L. F. Kebler (Bureau of Chemistry, Washington, D. C.), *Chairman.*
H. C. Lythgoe, Boston, Mass.
H. C. Fuller, Washington, D. C.
A. R. Bliss, Emory University, Ga.
W. S. Hubbard, New York, N. Y.

*Recommendations of Referees.*

(Figures in parenthesis refer to year in which appointment expires.)

R. E. DOOLITTLE (Transportation Building, Chicago, Ill.), *Chairman.*

SUBCOMMITTEE A: B. B. Ross (1926), (Polytechnic Institute, Auburn, Ala.), *Chairman,* W. H. MacIntire (1924), C. C. McDonnell (1922). [Fertilizers (borax in fertilizers, preparation of ammonium citrate, nitrogen, potash, potash availability, precipitated phosphates), inorganic plant constituents, (sulfur and phosphorus in the seeds of plants, calcium and magnesium in the ash of seed), water, tanning materials and leather, insecticides and fungicides, and soils (sulfur in soils).]

SUBCOMMITTEE B: H. C. Lythgoe (1926), (State Department of Public Health, Boston, Mass.), *Chairman,* E. M. Bailey (1924), C. A. Browne (1922). [Foods and feeding stuffs (crude fiber, stock feed adulteration), saccharine products (honey, maple products, maltose products, sugar-house products), dairy products (moisture in cheese, cryoscopic examination of milk, methods for fat in malted and dried milk), fats and oils, baking powder (fluorides in baking powder), drugs (examination of arsphenamine and neoarsphenamine; determination of alcohol in drug preparations; analytical methods for the determination of chloral hydrate in drug preparations; analytical methods for the determination of silver in silver proteinates; determination of camphor in pills and tablets by the alcohol distillation method; distillation method for the estimation of santalol in santal oil; turpentine; crude drugs; alkaloids; methods of analysis of morphine, codeine and diacetylmorphine; laxative and bitter tonic drugs; the determination of calomel, mercuric chloride and mercuric iodide in tablets; the analysis of acetylsalicylic acid; methods for the examination of phenolphthalein; method for the analysis of monobromated camphor; methods for the separation and estimation of the principal cinchona alkaloids; differentiation of Japanese and American peppermint oils), testing chemical reagents, nonalcoholic beverages, and eggs and egg products.]

SUBCOMMITTEE C: R. E. Doolittle (1926), (Transportation Building, Chicago, Ill.), *Chairman,* W. C. Geagley (1924), W. W. Randall (1922). [Food preservatives (saccharin), coloring matters (oil-soluble colors), metals in foods (arsenic), pectin in fruits and fruit products, moisture in dried fruit, canned foods, cereal foods, limit of accuracy in the determination of small amounts of alcohol in beers, vinegars, flavoring extracts, meat and meat products (separation of meat proteins, decomposition of meat products, gelatin), spices, determination of shells in cacao products, methods for the examination of cacao butter, coffee, tea, and nitrogen in foods.]

*Board of Editors.*

R. W. Balcom (Box 290, Pennsylvania Avenue Station, Washington, D. C.), *Chairman.*

C. B. Lipman (1922). William Frear (1924).
R. E. Doolittle (1923). R. B. Deemer (1925).
Marian E. Lapp, *Associate Editor.*

*Editing Methods of Analysis.*

R. E. Doolittle (Transportation Building, Chicago, Ill.), *Chairman.*

B. B. Ross. J. W. Sale.
A. J. Patten. G. W. Hoover.
W. H. MacIntire.

## Special Committees.

*Vegetation Tests on the Availability of Phosphoric Acid in Basic Slag.*

H. D. Haskins (Agricultural Experiment Station, Amherst, Mass.), *Chairman.*
C. B. Williams. B. L. Hartwell.
W. B. Ellett. J. A. Bizzell.

*Committee to Cooperate with the American Society for Testing Materials on the Subject of Agricultural Lime.*

W. H. MacIntire (Agricultural Experiment Station, Knoxville, Tenn.), *Chairman.*
William Frear. F. P. Veitch.

*Committee on Revision of Methods of Soil Analysis.*

C. B. Lipman (Agricultural Experiment Station, Berkeley, Calif.), *Chairman.*
W. H. MacIntire. R. Stewart.
A. W. Blair. J. A. Bizzell.

*Committee on Quartz-Plate Standardization and Normal Weight.*

Frederick Bates (Bureau of Standards, Washington, D. C.), *Chairman.*
C. A. Browne. F. W. Zerban.

*Representative to Cooperate with the Revision Committee of the United States Pharmacopœia.*

L. F. Kebler, Bureau of Chemistry, Washington, D. C.

*Representatives on the Board of Governors of the Crop Protection Institute of the National Research Council.*

B. L. Hartwell, Kingston, R. I.
H. J. Patterson, College Park, Md.

*Referees and Associate Referees.*

*Fertilizers:*
*Referee:* R. N. Brackett, Clemson Agricultural College, Clemson College, S. C.

*Borax in Fertilizers:*
*Associate referee:* J. M. Bartlett, Agricultural Experiment Station, Orono, Me.

*Preparation of ammonium citrate:*
*Associate referee:* C. S. Robinson, Agricultural Experiment Station, E. Lansing, Mich.

*Nitrogen:*
*Associate referee:* I. K. Phelps, Bureau of Chemistry, Washington, D. C.

*Potash:*
*Associate referee:* J. T. Foy, Clemson Agricultural College, Clemson College, S. C.

*Potash availability:*
*Associate referee:* A. G. McCall, Agricultural Experiment Station, College Park, Md.

*Precipitated phosphates:*
*Associate referee:* H. D. Haskins, Agricultural Experiment Station, Amherst, Mass.

*Inorganic plant constituents:*

*Referee:* A. J. Patten, Agricultural Experiment Station, E. Lansing, Mich.

*Sulfur and phosphorus in the seeds of plants:*

*Associate referee:* W. L. Latshaw, Agricultural Experiment Station, Manhattan, Kans.

*Calcium, magnesium, iron, and aluminium in the ash of seed:*

*Associate referee:* A. J. Patten, Agricultural Experiment Station, E. Lansing, Mich.

*Water:*

*Referee:* J. W. Sale, Bureau of Chemistry, Washington, D. C.

*Tanning materials and leather:*

*Referee:* F. P. Veitch, Bureau of Chemistry, Washington, D. C.

*Insecticides and fungicides:*

*Referee:* J. J. T. Graham, Bureau of Chemistry, Washington, D. C.

*Soils:*

*Referee:* W. H. MacIntire, Agricultural Experiment Station, Knoxville, Tenn.

*Sulfur in soils:*

*Associate referee:* W. H. MacIntire, Agricultural Experiment Station, Knoxville, Tenn.

*Foods and feeding stuffs:*

*Referee:* J. B. Reed, Bureau of Chemistry, Washington, D. C.

*Crude fiber:*

*Associate referee:* G. S. Fraps, Agricultural Experiment Station, College Station, Texas.

*Stock feed adulteration:*

*Associate referee:* H. E. Gensler, State Department of Agriculture, Harrisburg, Pa.

*Saccharine products:*

*Referee:* H. S. Paine, Bureau of Chemistry, Washington, D. C.

*Honey:*

*Associate referee:* S. F. Sherwood, Bureau of Plant Industry, Washington, D. C.

*Maple products:*

*Associate referee:* C. H. Jones, Agricultural Experiment Station, Burlington, Vt.

*Maltose products:*

*Associate referee:* O. S. Keener, Bureau of Chemistry, Washington, D. C.

*Sugar-house products:*

*Associate referee:* J. F. Brewster, Sugar Experiment Station, New Orleans, La.

*Dairy products:*

*Referee:* Julius Hortvet, State Dairy and Food Commission, St. Paul, Minn.

*Moisture in cheese:*

*Associate referee:* L. C. Mitchell, U. S. Food and Drug Inspection Station, 310 Federal Office Building, Minneapolis, Minn.

*Cryoscopic examination of milk:*

*Associate referee:* E. M. Bailey, Agricultural Experiment Station, New Haven, Conn.

*Methods for fat in malted milk and dried milk:*

*Associate referee:* J. T. Keister, Bureau of Chemistry, Washington, D. C.

*Fats and oils:*

*Referee:* G. S. Jamieson, Bureau of Chemistry, Washington, D. C.

*Baking powder:*

*Referee:* L. H. Bailey, Bureau of Chemistry, Washington, D. C.

*Fluorides in baking powder:*

*Associate referee:* J. K. Morton, Bureau of Chemistry, Washington, D. C.

*Drugs:*

*Referee:* G. W. Hoover, U. S. Food and Drug Inspection Station, Transportation Building, Chicago, Ill.

*Examination of arsphenamine and neoarsphenamine:*

*Associate referee:* G. W. Hoover, in collaboration with C. K. Glycart, U. S. Food and Drug Inspection Station, Transportation Building, Chicago, Ill.

*Determination of alcohol in drug preparations:*

*Associate referee:* A. G. Murray, Bureau of Chemistry, Washington, D. C.

*Determination of chloroform in drug preparations:*

*Associate referee:* A. G. Murray, Bureau of Chemistry, Washington, D. C.

*Methods for the examination of phenolphthalein:*

*Associate referee:* S. Palkin, Bureau of Chemistry, Washington, D. C.

*Analytical methods for the determination of silver in silver proteinates:*

*Associate referee:* W. L. Mitchell, Room 1012, U. S. Appraiser's Stores, New York, N. Y.

*Determination of camphor in pills and tablets by the alcohol distillation method:*

*Associate referee:* G. H. Arner, Room 1012, U. S. Appraiser's Stores, New York, N. Y.

*Determination of calomel, mercuric chloride and mercuric iodide in tablets:*

*Associate referee:* E. C. Merrill, United Drug Company, Boston, Mass.

*Crude Drugs:*

*Associate referee:* A. Viehover, Bureau of Chemistry, Washington, D. C.

*Alkaloids:*

*Associate referee:* A. R. Bliss, Emory University, Emory University, Ga.

*Laxative and bitter tonic drugs:*

*Associate referee:* H. C. Fuller, Institute of Industrial Research, Washington, D. C.

*Turpentine:*

*Associate referee:* J. O. Clarke, U. S. Food and Drug Inspection Station, U. S. Custom-house, Savannah, Ga.

*Distillation method for the estimation of santalol in santal oil:*

*Associate referee:* C. W. Harrison, U. S. Food and Drug Inspection Station, Park Avenue Building, Baltimore, Md.

*Methods for the separation and estimation of the principal cinchona alkaloids:*

*Associate referee:* E. O. Eaton, U. S. Food and Drug Inspection Station, U. S. Appraiser's Stores, San Francisco, Calif.

*Methods for analysis of morphine, codeine and diacetylmorphine:*

*Associate referee:* C. K. Glycart, U. S. Food and Drug Inspection Station, Transportation Building, Chicago, Ill.

*Analysis of acetylsalicylic acid:*

*Associate referee:* A. E. Paul, U. S. Food and Drug Inspection Station, Government Building, Cincinnati, Ohio.

*Methods for the examination of methylene blue:*

*Associate referee:* H. O. Moraw, U. S. Food and Drug Inspection Station, Transportation Building, Chicago, Ill.

*Methods for the examination of procaine:*

*Associate referee:* A. W. Hansen, U. S. Food and Drug Inspection Station Transportation Building, Chicago, Ill.

*Methods for the examination of pyramidon:*

*Associate referee:* A. W. Hansen, U. S. Food and Drug Inspection Station, Transportation Building, Chicago, Ill.

*Atophan:*

*Associate referee:* W. Rabak, U. S. Food and Drug Inspection Station, Federal Office Building, Minneapolis, Minn.

*Chloramine products:*

*Associate referee:* W. H. Heath, Food and Drug Inspection Station, Federal Building, Buffalo, N. Y.

*Testing chemical reagents:*

*Referee:* G. C. Spencer, Bureau of Chemistry, Washington, D. C.

*Non-alcoholic beverages:*

*Referee:* W. W. Skinner, Bureau of Chemistry, Washington, D. C.

*Eggs and egg products:* (To be filled later.)

*Food preservatives (saccharin):*

*Referee:* M. G. Wolf, U. S. Food and Drug Inspection Station, U. S. Appraiser's Stores, New York, N. Y.

*Coloring matters (oil-soluble colors):*

*Referee:* A. L. Burns, Old Customhouse, St. Louis, Mo.

*Metals in foods:*

*Referee:* W. F. Clarke, Bureau of Chemistry, Washington, D. C.

*Arsenic:*

*Associate referee:* R. M. Hann, Bureau of Chemistry, Washington, D. C.

*Pectin in fruits and fruit products:*

*Referee:* H. J. Wichmann, U. S. Food and Drug Inspection Station, Tabor Opera House Building, Denver, Colo.

*Moisture in dried fruit:*

*Referee:* R. W. Hilts, U. S. Food and Drug Inspection Station, U. S. Appraiser's Stores, San Francisco, Calif.

*Canned foods:*

*Referee:* R. W. Balcom, Bureau of Chemistry, Washington, D. C.

*Cereal foods:*

*Referee:* C. H. Bailey, Agricultural Experiment Station, University Farm, St. Paul, Minn.

*Limit of accuracy of small amounts of alcohol in beers:*

*Referee:* B. H. Hartmann, U. S. Food and Drug Inspection Station, Transportation Building, Chicago, Ill.

*Vinegars:*

*Referee:* W. C. Geagley, State Dairy and Food Department, Lansing, Mich.

*Flavoring extracts:*

*Referee:* W. W. Skinner, Bureau of Chemistry, Washington, D. C.

*Meat and meat products:*

*Referee:* C. R. Moulton, University of Missouri, Columbia, Mo.

*Separation of meat proteins:*

*Associate referee:* C. R. Moulton, University of Missouri, Columbia, Mo.

*Gelatin:*

*Referee:* C. R. Smith, Bureau of Chemistry, Washington, D. C.

*Spices:*

*Referee:* A. E. Paul, U. S. Food and Drug Inspection Station, Transportation Building, Chicago, Ill.

*Determination of shells in cacao products:*

*Referee:* B. H. Silberberg, Bureau of Chemistry, Washington, D. C.

*Methods for the examination of cacao butter:*

*Referee:* W. F. Baughman, Bureau of Chemistry, Washington, D. C.

*Coffee:*

*Referee:* H. A. Lepper, Bureau of Chemistry, Washington, D. C.

*Tea:*

*Referee:* R. E. Andrew, Agricultural Experiment Station, New Haven, Conn.

*Nitrogen in foods:*

*Referee:* I. K. Phelps, Bureau of Chemistry, Washington, D. C.

## LIST OF MEMBERS AND VISITORS PRESENT, 1921 MEETING.

Aldrich, Elizabeth, Bureau of Chemistry, Washington, D. C.
Alexander, L. M., 1703 New York Avenue, N. W., Washington, D. C.
Almy, L. H., Bureau of Chemistry, Washington, D. C.
Anderson, M. S., Bureau of Soils, Washington, D. C.
Arner, G. H., U. S. Food and Drug Inspection Station, U. S. Appraiser's Stores, New York, N. Y.

Badger, C. H., Bureau of Chemistry, Washington, D. C.
Bailey, E. M., Agricultural Experiment Station, New Haven, Conn.
Bailey, H. S., Southern Cotton Oil Co., Savannah, Ga.
Bailey, L. H., Bureau of Chemistry, Washington, D. C.
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Balcom, R. W., Bureau of Chemistry, Washington, D. C.
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Ball, C. O., National Canners Association, 1739 H Street, N. W., Washington, D. C.
Barker, F. A., Bureau of Soils, Washington, D. C.
Barnes, J. W., Bureau of Chemistry, Washington, D. C.
Bates, Carleton, U. S. Glue Company, Milwaukee, Wis.
Bates, Frederick, Bureau of Standards, Washington, D. C.
Baughman, W. F., Bureau of Chemistry, Washington, D. C.
Beal, W. H., States Relations Service, Washington, D. C.
Bell, H. G., Canadian Fertilizer Association, Toronto, Canada.
Bentley, C. H., California Packing Corporation, San Francisco, Calif.
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Boyle, Martin, Bureau of Chemistry, Washington, D. C.
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Brown, B. E., Bureau of Plant Industry, Washington, D. C.
Buchanan, Miss Ruth, Bureau of Chemistry, Washington, D. C.
Bumgamer, A. J., Uniontown, Pa.
Burritt, Loren, Treasury Department, Washington, D. C.
Burroughs, L. C., State Department of Health, 16 W. Saratoga Street, Baltimore, Md.

Capen, Miss R. G., Bureau of Chemistry, Washington, D. C.
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Dallas, Miss M. L., Bureau of Chemistry, Washington, D. C.
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Davis, R. O. E., Bureau of Soils, Washington, D. C.
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Duvall, Miss Louise, Bureau of Chemistry, Washington, D. C.

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Ellis, N. R., Bureau of Animal Industry, Washington, D. C.
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Finks, A. J., Bureau of Chemistry, Washington, D. C.
Fitzgerald, F. F., American Can Company, Maywood, Ill.
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Foster, Miss M. D., U. S. Geological Survey, Washington, D. C.
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Frear, William, Agricultural Experiment Station, State College, Pa.
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Gebhart, A. I., Bureau of Internal Revenue, Washington, D. C.
Gensler, H. E., Department of Agriculture, Harrisburg, Pa.
Gersdorff, C. E. F., Bureau of Chemistry, Washington, D. C.
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Grille, G. A. Jr., Eimer & Amend, New York, N. Y.
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Grotlisch, V. E., Bureau of Chemistry, Washington, D. C.

Haller, H. L., Bureau of Chemistry, Washington, D. C.
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Hand, W. F., Agricultural College, Agricultural College, Miss.

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Harris, J. R., Tennessee Coal, Iron and Railroad Company, Birmingham, Ala.
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Hart, B. R., Department of Commerce, Washington, D. C.
Hart, Leslie, Bureau of Chemistry, Washington, D. C.
Hartman, Miss M. E., Bureau of Chemistry, Washington, D. C.
Haskins, H. D., Agricultural Experiment Station, Amherst, Mass.
Hayes, J. F., 8 Donaldson Street, Cherrydale, Va.
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Himmler, L. W., Bureau of Animal Industry, Washington, D. C.
Hodgins, R. J., University of Maryland, College Park, Md.
Holm, G. E., Bureau of Animal Industry, Washington, D. C.
Holman, H. P., Bureau of Chemistry, Washington, D. C.
Holmes, R. S., Bureau of Soils, Washington, D. C.
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Hortvet, Julius, State Dairy and Food Commission, St. Paul, Minn.
Houghton, H. W., Hygienic Laboratory, Washington, D. C.
Howard, B. J., Bureau of Chemistry, Washington, D. C.
Howard, C. S., U. S. Geological Survey, Washington, D. C.
Howe, H. E., Otis Building, Washington, D. C.
Hubbard, W. S., U. S. Food and Drug Inspection Station, U. S. Appraiser's Stores, New York, N. Y.
Hurd, W. D., 819 Southern Building, Washington, D. C.
Hurst, L. A., Bureau of Plant Industry, Washington, D. C.
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Irwin, W. H., Swift and Company, Chicago, Ill.

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# PRESIDENT'S ADDRESS[1].

## RECENT TENDENCIES OF RESEARCHES ON THE PHOTOSYNTHETIC PROCESSES OF PLANTS.

By W. F. Hand (Mississippi Agricultural and Mechanical College, Agricultural College, Miss.), *President.*

Throughout all the years of its life, this organization has held closely to its original purpose. The steady expansion of its activities is convincing evidence of a proper interpretation of its mission, and even now its interests are so varied and far-reaching that we fear that we must soon look forward to the time when we will be compelled to sacrifice something of solidarity and good fellowship by the adoption of the group system in conducting our annual convocations. But however that may be, we are certain to become increasingly useful. From our constant excursions into the unknown, we have brought back during the many years of our history much knowledge useful for the daily guidance and essential for the future development of the industrial and

[1] Presented Tuesday morning, October 25, 1921, as special order of business for 11 o'clock.

governmental activities with which we are closely related. In this respect we may believe that we have been particularly favored by fortune; for to the mere pleasure arising from co-operative study there can be added the satisfying assurance that our labors have been distinctly helpful to our day and generation.

In earlier times especially there was a pressing need for a quick turnover. The necessity for close association and concerted attack on important problems drew forth all possible energy. It may be that we are destined to become accustomed to mass production in scientific research in general as a result of unified administration and specialization. If that be true, we shall not be wholly unprepared for the new order.

But while we are devoting the closest attention to the advancement of the work lying nearest at hand, the ideal of immediate effective service will not be pursued with so much zeal that no time and no interest will remain for reflection upon great and even majestic problems in our field, the final solution of which will demand the life-labor of generations, perhaps, of able, unselfish men. I have believed it not inappropriate, therefore, to endeavor to direct your attention for a short while to some of the aspects of a distinctly fundamental biochemical problem.

From the earliest times men have been puzzled by the varied and manifold phenomena of life. If even the processes of the simpler plant forms, which are indefinitely less complex than those of animals, could be wholly elucidated, a yearning of many centuries would be satisfied. We are slowly, but, let us hope, none the less surely, approaching the day of that realization. An imposing collection of facts has become available, and we take encouragement from the belief that the greatest difficulties yet remaining shall not overwhelm us.

More than two hundred years ago it was known that leaves were in some way connected with the elaboration of tissue, but Priestley, in 1772, was the first to show that plants restored respired air. He was rewarded with a medal in recognition of the importance of his discovery, but his investigations were interrupted somewhat by the publication of the experimental researches of the Dutch physician, Ingenhousz, who received no thanks from Priestley for this unsolicited assistance. Ingenhousz established beyond doubt that leaves evolve oxygen in light and spoil air in darkness.

But to de Saussure belongs the credit of first demonstrating that plants may take the carbon required for their development from the atmosphere. Liebig was not slow in appreciating the fundamental nature of the discoveries resulting from de Saussure's studies, but he was not always wholly clear with regard to some of the aspects of plant respiration. Boussingault, having much better apparatus available, fully confirmed de Saussure's work and pointed out in a very con-

vincing way that the synthesis of sugar was, after all, the goal of carbon dioxide assimilation.

Many men whose names are eminent in our literature have made contributions to the still unrevealed method of the primary synthetic processes occurring in plant leaves. Accounts of the historical development of the subject are easily available. The effort that has been expended is truly prodigious; it is surely deserving of a better outcome.

Numerous vexing questions have arisen to beset the investigator. In the first place, the minute amount of carbon dioxide in the atmosphere, the small total area of the openings in leaves and the large amount of carbon in plants have made the rapid growth of vegetation appear not a little mysterious to those giving no special study to the subject. A square centimeter of leaf surface may take up during a single hour an amount of carbon dioxide equivalent to that in a column of air of the same cross section and more than one hundred centimeters long. The avidity with which leaves take in $CO_2$ is truly surprising. Liebig compared them in this respect to a paste of calcium hydroxide, and Brown[1] has shown that the analogy is by no means unjustified, because he found that a strong solution of caustic alkali, contained in a special apparatus providing for constant renewal, could absorb 0.17 cc. of $CO_2$ an hour for each square centimeter. A catalpa leaf has been observed to take up $CO_2$ more than half as rapidly as that. Brown has pointed out also the true explanation of the way in which so large an amount of $CO_2$ may pass into the stomata. Computations show that only the slightest difference in the partial pressure of the gas within and without the leaf is quite sufficient to account for the rapid exchange required in the absorption.

The literature has accumulated an impressive array of hypotheses and theories with reference to the mechanism of carbon fixation. A few of these are little more than improbable suggestions resting on no experimental work; others derive error from faulty experiment or from improper deductions, and the question is still awaiting an answer, definite and direct. There is no occasion to exhaust your patience by even a brief discussion of much of the important work that has been accomplished in this field. The older researches, however, may not be lightly dismissed, because these have made the way less difficult for the more careful studies of recent years.

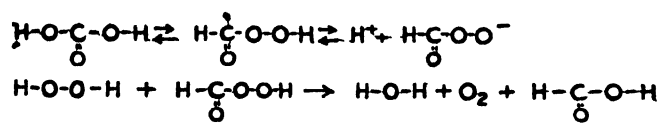
One finds almost everywhere in the literature reference to the historic suggestion of A. Baeyer[2] that formaldehyde is an intermediate product of $CO_2$ reduction in the leaf. Carbohydrates may result through aldol condensations and by the loss of water from the condensed nuclei. The

[1] *Proc. Roy. Institution (Great Britain)*, 1901, **16**: 547.
[2] *Ber. Chem. Ges.*, 1870, **3**: 63.

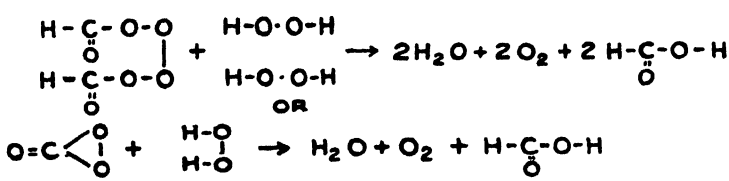
theory appeared to be a very happy one, and almost no end of labor has been put forth in unsuccessful endeavors to fully confirm it. Failing in this, and being unable to ascertain the relation of chlorophyll to the process, researchers have looked in other directions for theories; and perhaps it was in despair that some of the rather unique hypotheses which have come forth have had their birth.

Wislicenus[1] has recently carried out the reduction of carbonic acid to formic acid by hydrogen peroxide and also by the electrolysis of a solution of potassium bicarbonate. He has sought to build a theory of carbon assimilation on the basis of these results. He suggests that there is a sufficient amount of $H_2O_2$ in the air to carry out the process in nature. The formic acid produced in the reduction (the reaction requires no energy absorption) is subsequently converted to formaldehyde, the energy increment being received through light absorption by aid of chlorophyll catalysis.

The explanation of the reduction of $H_2CO_3$ by $H_2O_2$ to formic acid requires a postulate with reference to the structure of the anion of the acid. It is assumed that an aqueous solution of it must contain some concentration of percarbonic acid in order that reduction may be accomplished by the peroxide. The reaction follows:



During electrolysis of a solution of $KHCO_3$, the $H_2O_2$, formed by discharge of two OH ions, may react with the discharged anion of peroxide structure, reduction then being accomplished by splitting out of oxygen. Omitting the cations, the anodic reduction may be expressed by

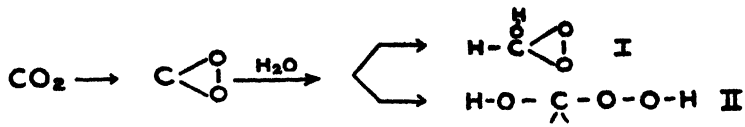


In the leaves of plants the formic acid is reduced to formaldehyde by the aid of chlorophyll and through the absorption of the necessary energy from light.

In this cycle of changes, the volume of $CO_2$ converted to formic acid is identical with that of the oxygen evolved. This ratio corresponds with the best observations that have been made with growing leaves.

[1] *Ber. Chem. Ges.*, 1918, **51**: 942.

As unusual as the peroxide structure of carbonic acid may appear, the conception is not without its supporters. Woker[1] also believes it probable that the reactivity of $CO_2$ in the plant world is due to its ability to produce, in some measure at least, unstable isomers through combination with water, in accordance with the following representations:




The unstable peroxide, II, would tend to lose oxygen, leaving a methylene radical, isomeric with formaldehyde, and conceivably prone to enter into numerous condensations resulting in the production of such bodies as dihydroxyacetone, glucose, furfural, and so forth.

Woker looks upon chlorophyll as taking possibly the role of a sensibilizer of the peroxide isomerization of carbonic acid, and also as an intermediate catalyzer of the condensation phases of the subsequent reactions. The optical activity of chlorophyll would permit the production of optically active compounds.

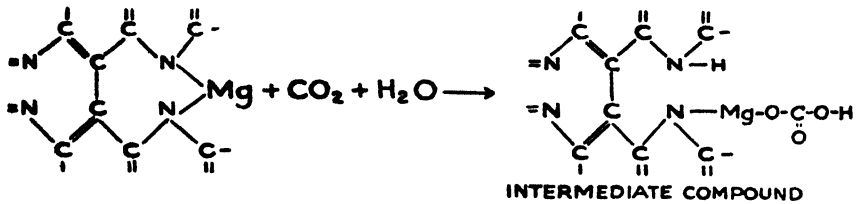
Without attempting to detract in the least from the plausibility of Woker's cycle, it may be added that a heavy burden of proof is again placed upon the chlorophyll molecule. This is not unusual in researches in this field. Charges and countercharges have been brought against the green coloring matter of the plants. Too sensitive not to feel the injury of unjust accusation, but suffering in silence, it is truly a stoic in the molecular world.

No lesser authorities than Willstätter and Stoll[2] also bring forth a theory of photosynthetic action involving a shifting of valence in the carbonic acid molecule as a result of the absorption of radiant energy. According to this view, carbonic acid may pass over to formylhydrogen peroxide, or to performic acid. The rearrangement of the latter to carbonic acid is already well known. It appears possible that a derivative of formaldehyde peroxide may also have a share in the scheme of assimilation which the authors suggest, and this conception would carry still greater force but for the fact that the compound itself remains undiscovered.

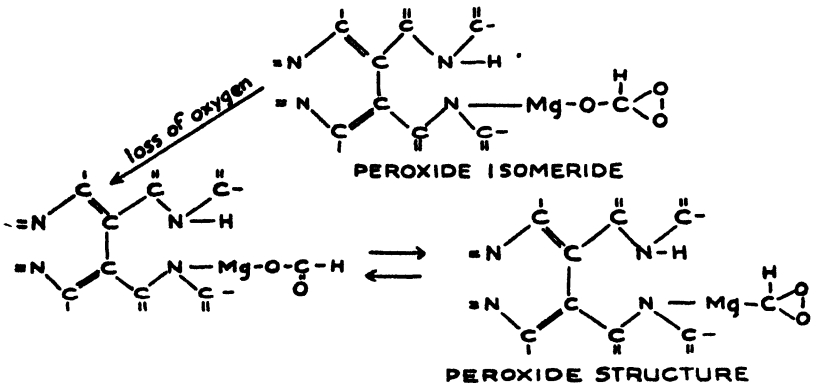
Willstätter and Stoll show that aqueous colloidal solutions of chlorophyll (but not dispersions in organic liquids) take up $CO_2$ from the air. Such solutions are capable of reacting with two molecules of $H_2CO_3$, the reaction being complete when $Mg(HCO_3)_2$ is split off. In the

[1] *Arch. ges. Physiol.*, 1919, 176: 11; *Chem. Abstr.*, 1920, 14: 963.
[2] *Ber. Chem. Ges.*, 1917, 50: 1791.

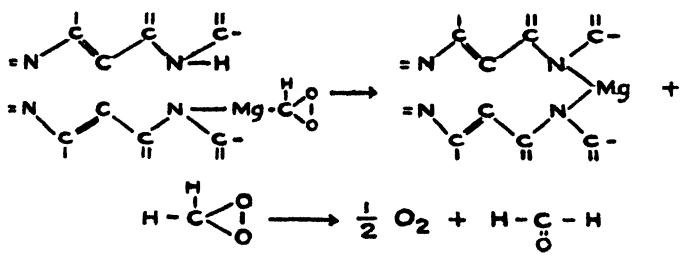
course of these changes an intermediate compound of chlorophyll and $H_2CO_3$ may be produced, and this is dissociable:




If the intermediate body is imagined to rearrange into the isomeric peroxide structure and then to lose oxygen, it would be natural to expect the production of a chlorophyll compound of formic acid:



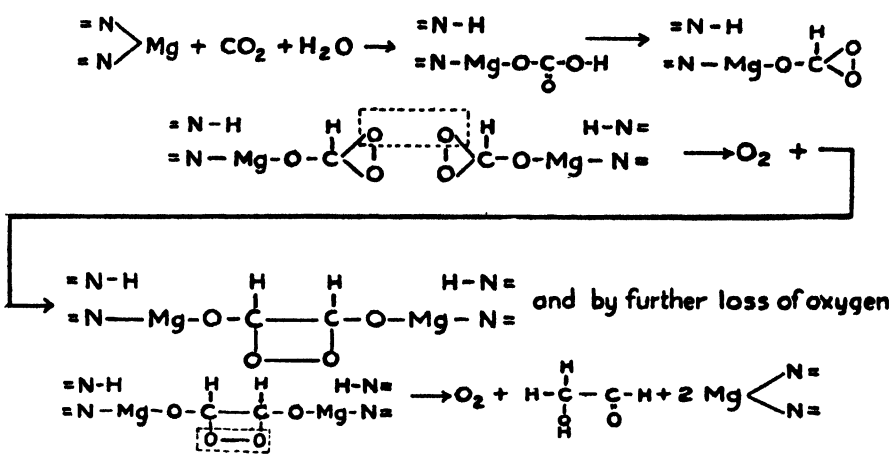

The formate is incapable of losing oxygen, but this does not apply to its corresponding peroxide isomeride, which parts with a half molecule, leaving a molecule of formaldehyde and reproducing the original chlorophyll structure:



Thus all of the $CO_2$ is accounted for, an equivalent volume of oxygen is liberated, and the often-mentioned progenitor of the carbohydrates, formaldehyde, is obtained.

A somewhat similar interpretation of the mechanism of carbon assimilation has been brought out recently by Rouge[1] who holds the opinion that glycolaldehyde is the first product produced in the assimilatory process. He shows that minute amounts of the aldehyde may be detected by *p*-nitrophenylhydrazine, and succeeds in making quantitative determinations by weighing the hydrazone. It is presumed that other aldehydes that may be present were not included in his qualitative tests and quantitative determinations.

Through a modification of the theory of Willstätter and Stoll, it is possible to account for the formation of the glycolaldehyde, the presence of which in plants Rouge believes his experiments to have demonstrated. The changes involved may be expressed as follows:

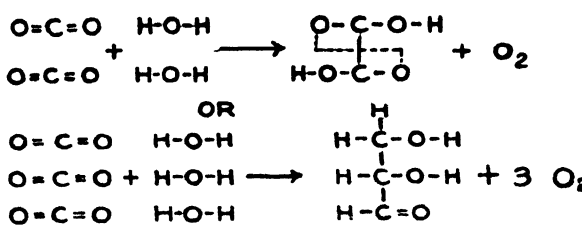



The co-operation of some unknown catalase is thought to be essential to a realization of these reactions. Perhaps there is little cause to question the plausibility of this view of carbohydrate production, but certainly there is reason to hope that experimental evidence will continue to accumulate until we may judge its merits more clearly than we are permitted to do to-day.

The desperate condition in which we find ourselves with reference to the forces and materials employed in the tiny laboratories of leaves is indicated by the bold guesses scattered throughout the voluminous literature of this very old subject. Nor are these suggestions, unsupported by experiments as many of them are, entirely without value. We have not found the way, but we can search the better for it with the aid of the light we already possess. The cost of failures and of trials will not remain long in memory when we are finally rewarded by successful accomplishment.

[1] *Schweiz. Apoth.—Ztg.*, 1921, 59: 157, 175; *Chem. Abstr.*, 1921, 15: 2294.

Finding that the diketone, benzil and hydrogen react in light to form benzoin, Kögel[1] has thought that the reaction under these conditions might form a pattern for photosynthetic assimilation. He conceives the possible formation of an intermediate dihydroxyethylene dioxide through the reaction of carbon dioxide and water, and the subsequent breaking up of this body into formaldehyde and oxygen, or the production from it of formic or oxalic acid. In a similar way glyceraldehyde and oxygen may be a result of interaction of $CO_2$ and $H_2O$ under external influences. The compounds so produced are suited to carbohydrate synthesis:



Investigators in general have become thoroughly indoctrinated with the chlorophyll theory; it is regarded as the material agent mostly responsible for the strange transformations which occur in the building up of organic matter. Preponderating evidence, direct and indirect, appears to favor this view, but it can scarcely be said that its experimental demonstration has been accomplished.

Moore[2], whose excellent work will be praised by all students of carbon assimilation, points out the absence of proof that the colorless parts of the chloroplasts may not be the seat of the synthetic activities. Chlorophyll cannot be developed in absence of iron, though the green itself is free of iron. Inorganic iron in crystalloidal or colloidal states is a constant component of the colorless parts of chloroplasts; and, therefore, it appears that chlorophyll is itself a photosynthetic product formed through the agency of the iron-bearing parts of the leaf structure. It is Moore's opinion that the parts containing iron and the chlorophyll produced by them become associated in photosynthesis and form a complete mechanism for the energy transformations of plant growth.

From his study of the photoöxidation of chlorophyll and xanthophyll, Ewart[3] was led to suggest a rather unique hypothesis with reference to the photosynthetic fixation of carbon. He thinks of the process as taking place essentially in three stages involving:

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[1] *Zeit. wiss. Phot.*, 1920, **19**: 215; *Chem. Abstr.*, 1920, **14**: 3440.
[2] Proc. Roy. Soc. (London) 1914, **87***B*: 556.
[3] Ibid., 1917, **89***B*, 1.

(1) The absorption of carbon dioxide and water by the "phytyl base" of chlorophyll, forming xanthophyll as an intermediate product; (2) the oxidation of xanthophyll to the original "phytyl base", hexose sugars, and formaldehyde, the excess of oxygen accumulated (equivalent to the volume of $CO_2$ originally taken up) in state (1) being liberated; (3) the return of the "phytyl base" to the chlorophyll molecule and the production of carbohydrates from the formaldehyde.

These possibilities put forth by Ewart are quite original and will be considered as bold, perhaps, by students of the question, The cycle can be understood from an inspection of the equations:

$$(1)\quad \underset{\text{"PHYTYL BASE"}}{4\,C_{20}H_{39}COO} + 76\,CO_2 + 34\,H_2O \rightarrow \underset{\text{XANTHOPHYLL}}{4\,C_{40}H_{56}O_2} + 93\,O_2$$

$$(2)\quad 4\,C_{40}H_{56}O_2 + 42\,H_2O + 93\,O_2 \rightarrow 4\,C_{20}H_{39}COO + 8\,C_6H_{12}O_6 + 28\,HCHO + 76\,O_2$$

$$(3)\quad \text{RETURN OF "PHYTYL BASE" TO CHLOROPHYLL MOLECULE, } C_{21}H_{29}N_4Mg\left\{\begin{array}{l}-COOH\\-COOCH_3\\-COOC_{20}H_{39}\end{array}\right.$$

The way in which radiant energy is appropriated by plants still remains to supply cause for much additional work. The partial reduction of carbon dioxide by silent electric discharges, by discharges under reduced pressure, and reduction of carbonates by electrolysis have probably suggested the possibility of electric reduction in the chloroplasts. But such a theory is of little help because through it we apparently pass simply from one step in the unknown to another.

But in this connection Noack[1] points out facts that are not without interest. Fluorescent substances, *e. g.*, eosin, fluorescein, methylene blue, etc., act in light as peroxides on plant chromogens. There are, therefore, light catalyzers. The peroxides come to equilibrium with atmospheric oxygen. In presence of an oxygen carrier like a manganese salt, the photodynamic oxidation becomes very powerful. Chlorophyll in this way may be converted into a peroxide capable of isomerizing the bicarbonate to a group suitable for reduction; whereby oxygen is evolved and the original fluorescent color substances again produced. Fluorescent bodies do not act in the same way, however, as metallic light catalyzers, and, therefore, it must be true that the conversion of radiant energy by these colors is different from transformations of chemical energy of the kind on which catalyzers ordinarily depend.

Moore and Webster[2] have demonstrated the production of organic matter from carbonic acid solutions containing ferric or uranic hydroxide. The photosynthesis took place in ordinary light. Baly, Heilbron and Barker[3] have confirmed these results. They show that other colored

[1] *Z. Botan.*, 1920, **12**: 237; *Chem. Abstr.*, 1921, **15**: 2453.
[2] *Proc. Roy. Soc.*, 1914, **87***B*: 163.
[3] *J. Chem. Soc.*, 1921, **119**: 1025.

substances, *e. g.*, malachite green, methyl orange, and so forth, will produce the same effect. The latter authors have also proved that formaldehyde and carbohydrates may be obtained from carbonic acid solutions by exposure to ultra-violet light. No catalyzer is required. The very short wave lengths bring about the reduction to formaldehyde and the longer oscillations catalyze its condensation to reducing sugar.

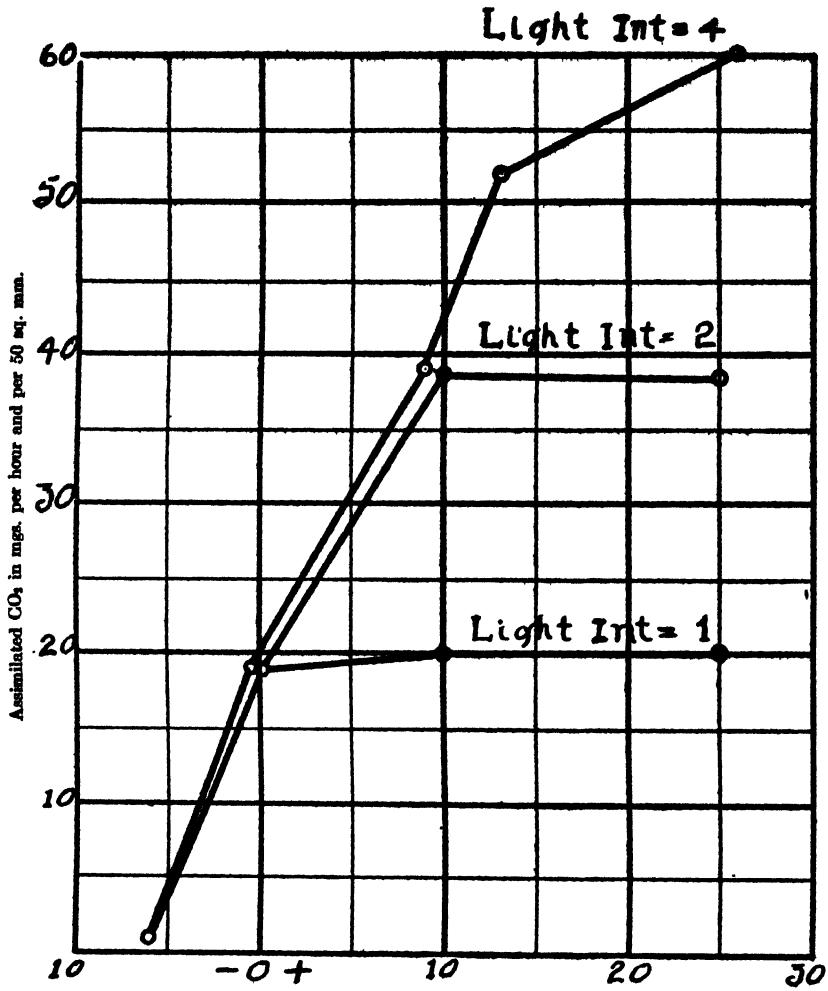
But plants grow in light containing only a very small proportion of short waves. A photocatalyst is, therefore, essential. Such a substance must be capable of absorbing light of longer wave-length than $350\mu\mu$ and of then radiating this energy at infra-red frequencies. The condensation of formaldehyde is favored by longer wave-lengths than those which induce its formation. The production of carbohydrates, according to these views, must occur in two stages; and these investigators look upon chlorophyll as the ideal photocatalyst for both.

When one reflects upon the vast amount of energy stored away in coal, oil and gas fields and the great quantity being recovered in the cycle of the seasons, the inefficiency of the agencies that gather it is very surprising. Brown and Escombe[1] in a wonderfully complete system of measurements, obtained data that cause us to hope that the methods now in use in leaf laboratories will undergo gradual improvement as the demands of increasing animal life draw more heavily year by year upon the products of their manufacture. The incident energy is disposed of about as follows:

| | Per Cent |
|---|---|
| Energy used for photochemical work | 00.66 |
| Energy used for transpiration | 48.39 |
| Solar radiant energy transmitted | 31.40 |
| Energy lost by thermal emission | 19.55 |
| Total | 100.00 |

The factors which govern most largely the speed of the assimilatory process are naturally intensity of illumination, concentration of $CO_2$, temperature and number of stomata. Blackman and his students have given us most of our information regarding the relation of temperature to speed assimilation. Matthaei[2] has made careful measurements which developed an interesting relationship between intensity of illumination, temperature and amount of $CO_2$ assimilation. The general results are brought together in the diagram which follows, (I).

[1] *Proc. Roy. Soc. (London)*, 1905, 76*B*: 29, 137.
[2] Phil. Trans., 1905, 197: 48.

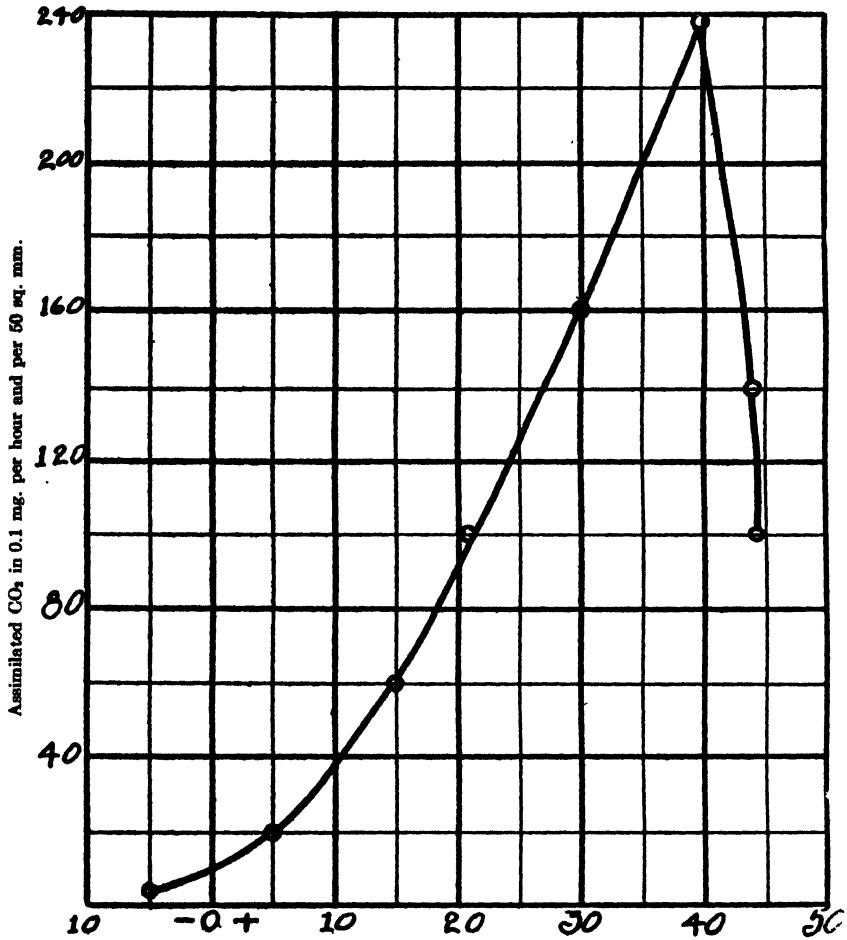



I. Relation of Temperature and Intensity of Illumination to Assimilation, after Matthaei.

The studies of Matthaei emphasize also the marked influence of temperature upon the rate of intake of $CO_2$ (Diagram II). In the plants under investigation, the rate of carbon fixation increased rapidly with temperature rise up to the point at which damage to the tissue occurred. This is plainly brought out in the steep rise and sudden fall of the curve (Diagram II). It would prove highly interesting to know whether a study of numerous kinds of plants would result in similar conclusions.

Blackman and Smith[1] with improved apparatus extended their studies

[1] *Proc. Roy. Soc. (London)*, 1910, 83*B*: 374.



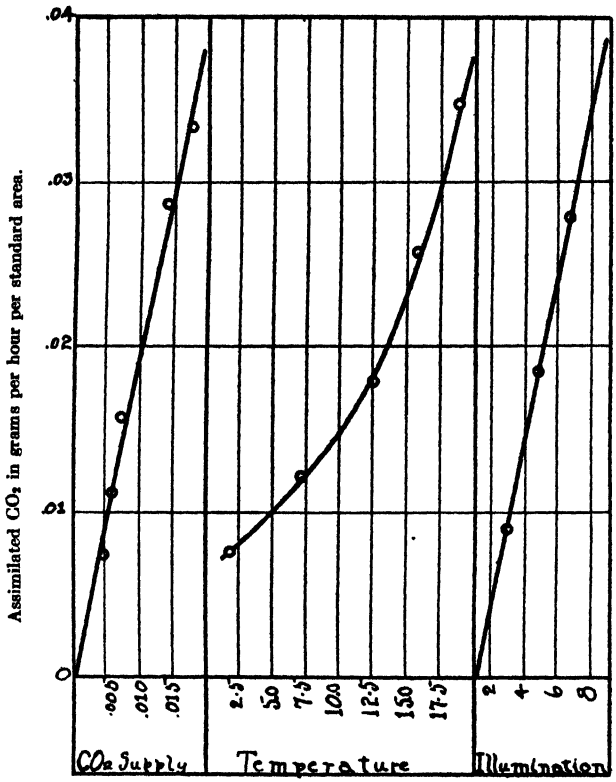

II. Influence of Temperature on Assimilation, after Matthaei.

to water plants. In the case of *Elodea* the data enabled them to show in a very instructive way the inter-relationship of $CO_2$ concentration, temperature and intensity of illumination (Diagram III). An extension of these studies to several kinds of land and water plants would supply highly desirable information.

The graphic representation of the relationships developed is plain enough to require no discussion.

When we think of the present state of our information with reference to the assimilation of carbon and reflect upon the wealth of labor which has very naturally been lavished upon a problem so fundamental, there is need to call to mind the fact that the spirit of scientific inquiry permits of no thought of ultimate defeat. Those of this and other generations who are enabled to interpret that spirit truly possess the

vision and hold the faith that will conquer. Let us have every assurance that in this difficult contest, long though it may prove to be, Nature will lose.




III. Influence of different factors on assimilation in a water plant, *Elodea*, after Blackman and Smith.

## FIRST DAY.

# MONDAY—MORNING SESSION.

The thirty-seventh annual convention of the Association of Official Agricultural Chemists was held at the Washington Hotel, Washington, D. C., October 24–26, 1921, about a month earlier than usual. It was found that it would be impossible to secure hotel accommodations during the month of November when the Conference for the Limitation of Armaments was in session.

The meeting was called to order by the President, W. F. Hand of Agricultural College, Miss., on the morning of October 24, 1921, at 10 o'clock.

## REPORT ON WATER.

By J. W. SALE (Bureau of Chemistry, Washington, D. C.), *Referee.*

During the past year the Weszelszky method[1] for the determination of iodine and bromine was studied in the Water and Beverage Laboratory by W. E. Shaefer, under the direction of the referee.

Briefly, the Weszelszky method depends upon the selective oxidation of bromine in acid solution by chlorine water, whereby bromine is set free, separated from the iodine by distillation in a stream of carbon dioxide, and absorbed in a bulb containing a solution of potassium hydroxide. The iodine, converted into iodic acid in the reaction flask, is titrated with standard sodium thiosulfate after the addition of some potassium iodide. The bromine converted into bromate in the absorption bulb and freed from chlorate by boiling carefully to dryness over a free flame is likewise titrated with standard sodium thiosulfate.

The kind and quantity of absorbing alkali and the time and temperature used to remove the chlorate were varied until satisfactory conditions for the recovery of bromine from bromine water were found. A modified absorption apparatus was constructed, and the kind and concentration of the acid added to the reaction flask were varied in an effort to recover bromine quantitatively from potassium bromide and estimate it by the method found to be satisfactory. Iodine was converted into iodic acid by chlorine water in the reaction flask and estimated in solutions of various acid concentrations.

A rapid and satisfactory modified Weszelszky method for the determination of small amounts of iodine based on these experiments was de-

[1] *Z. anal. Chem.*, 1900, 39: 81.

veloped. It was concluded, however, that the Weszelszky method for bromine in the presence of iodine, however modified, is incapable of giving satisfactory results on small samples, and its use is not recommended. Since it is proposed to recommend the method for iodine as an additional tentative method, some results obtained by its use in the Water and Beverage Laboratory are given below in Tables 1 and 2, Experiments 55–66. The data in these tables are sufficient also to show that the method for bromine is unsatisfactory.

TABLE 1.

*Results of tests for iodine and bromine using Weszelszky apparatus but modified method*.*

| EXPERIMENT† NO. | 10% POTASSIUM HYDROXIDE | CHLORINE WATER | IODINE PRESENT AS POTASSIUM IODIDE | IODINE FOUND | BROMINE PRESENT AS POTASSIUM BROMIDE | BROMINE FOUND |
|---|---|---|---|---|---|---|
| | *cc.* | *cc.* | *mgs.* | *mgs.* | *mgs.* | *mgs.* |
| 55 | 10 | 50 | 10.00 | 10.74 | 10.00 | 9.35 |
| 56 | 10 | 50 | 10.00 | 10.26 | 10.00 | 8.83 |
| 57 | 10 | 50 | 20.00 | 20.00 | 20.00 | 14.80 |
| 58 | 10 | 50 | 5.00 | 5.13 | 5.00 | 4.07 |
| 59 | 10 | 50 | 20.00 | 19.87 | 20.00 | 17.13 |

*50 cc. of chlorine water+5 cc. of 1 to 1 hydrochloric acid used in reaction flask. Samples heated in oven for 1 hour.

†Temperature of oven for Experiments 55, 56, and 57 was 152°-180°C.; that for Experiments 58 and 59 was 145°-169°C.

TABLE 2.

*Results of tests for iodine and bromine using Weszelszky method but modified apparatus*.*

| Experiment No. | ALKALI FOR ABSORPTION† | | | | IODINE PRESENT | IODINE FOUND | BROMINE PRESENT | BROMINE FOUND |
|---|---|---|---|---|---|---|---|---|
| | 1ST BOTTLE | | 2ND BOTTLE | | | | | |
| | 10% Solution potassium hydroxide | 10% Solution potassium carbonate | 10% Solution potassium hydroxide | 10% Solution potassium carbonate | | | | |
| | *cc.* | *cc.* | *cc.* | *cc.* | *mgs.* | *mgs.* | *mgs.* | *mgs.* |
| 60 | 7 | .. | 3 | .. | 10.00 | 10.06 | 10.00 | 5.84 |
| 61 | 7 | .. | 3 | .. | 10.00 | 9.97 | 10.00 | 5.61 |
| 62 | 10 | .. | 5 | .. | 5.00 | 5.05 | 5.00 | 3.93 |
| 63 | 10 | 35 | 10 | 35 | 10.00 | 10.15 | 10.00 | 6.88 |
| 64 | 10 | 35 | 10 | 35 | 10.00 | 10.07 | 10.00 | 7.18 |
| 65 | 22½ | 22½ | 22½ | 22½ | 10.00 | 10.13 | 10.00 | 2.85 |
| 66 | 22½ | 22½ | 22½ | 22½ | 10.00 | 10.06 | 10.00 | 7.74 |

*Kind and concentration of absorbing alkali varied. 5 cc. of 1 to 1 hydrochloric acid and 50 cc. of chlorine water were added to the reaction flask. Samples heated in oven for 1 hour.

†The amounts of alkali were added to the absorption bottles and diluted to 150 cc.

The results obtained on Experiments 57–66, in Tables 1 and 2, show that this method gives very satisfactory results for iodine provided

5 cc. of 1 to 1 hydrochloric acid is used in the reaction flask. The average error on 10 consecutive iodine determinations on samples of 5 to 20 mgs. (Experiments 57–66) was only 0.081 mg. or 0.74 per cent. The results of Experiments 55 and 56 are omitted from this average because they were the first ones made, and the solution in the reaction flask was not boiled sufficiently long to remove all the chlorine. The average error made by W. F. Baughman[1] on seven consecutive determinations of iodine on samples of 20.1 to 60.2 mgs. by the permanganate method was 0.229 mg. or 0.66 per cent.

The modified method for iodine follows:

## IODINE IN THE PRESENCE OF CHLORINE AND BROMINE.

### APPARATUS.

Glass-stoppered flask of 200–400 cc. capacity provided with inlet and outlet tubes. Tall absorption bottle.

### REAGENTS (1 to 1)

(a) *Hydrochloric acid (1 to 1).*
(b) *Chlorine water freshly prepared.*
(c) *10% potassium carbonate solution.*
(d) *Potassium iodide solution, 20% free from iodate.*
(e) *0.05N sodium thiosulfate solution.*
(f) *Starch solution.*

### DETERMINATION.

Place sample, contained in a volume of not more than 25 cc., in the glass-stoppered flask. The inlet tube should have a stop-cock and reach nearly to the bottom of the flask. Add 5 cc. of 1 to 1 hydrochloric acid to the sample, insert the stopper and add 50 cc. of freshly prepared chlorine water through the inlet tube. Place the outlet tube in a tall bottle containing 35 cc. of a solution of potassium carbonate (c) diluted to 150 cc., heat the reaction flask and boil gently until most of the chlorine and bromine has been distilled into the alkali. It is convenient to have a 25 cc. bulb blown into the middle of the outlet tube in order to lessen the danger of the absorbing solution running back into the reaction flask. Connect the inlet tube to a carbon dioxide generator and complete the distillation by simultaneous boiling and bubbling of carbon dioxide through the sample. Continue this for 10 minutes, testing for presence of chlorine and bromine by holding a piece of starch iodide paper at the end of the outlet tube; remove the source of heat, and bubble carbon dioxide through the apparatus until it is cool. Add 5 cc. of a solution of potassium iodide (d), and titrate the liberated iodine with 0.05N sodium thiosulfate solution (e) in the usual way.

Methods for the quantitative determination of small quantities of lead, copper and zinc in waters, when all three metals together with iron are present, have been collated. Synthetic samples have been analyzed by these methods, but additional work is needed before the methods can be accepted as completely satisfactory. Methods of water analysis

[1] *J. Ind. Eng. Chem.*, 1919, **11**: 563.

are incomplete unless methods for metals which are frequently sought are included. While methods which involve electrolytic deposition of metals are no doubt satisfactory, it is not believed that the average laboratory is equipped to make the determinations in this way; consequently, the methods which have been collated and in part tested are colorimetric. In the following methods the coloring matter is eliminated by precipitating the metals with ammonium sulfide. The lead is separated as lead sulfate and determined colorimetrically as lead sulfide. The copper is separated as copper sulfide and determined colorimetrically with potassium ferrocyanide. In the filtrate from the copper, the iron is held in solution with citric acid. The zinc is precipitated as zinc sulfide and determined turbidimetrically with potassium ferrocyanide.

The methods in detail follow:

### LEAD, COPPER AND ZINC (Quantitative)[1], [2].

(Use when water contains small quantities of metals.)

#### REAGENTS (SPECIAL).

(a) *Ammonium acetate solution.*—Dissolve 200 grams of the salt in water and make up to 500 cc.

(b) *Dilute ammonium acetate solution.*—Dilute 50 cc. of (a) to 500 cc.

(c) *3.5% potassium ferrocyanide solution.*

(d) *Standard lead solution.*—Add sulfuric acid in slight excess to a strong solution of lead acetate. Filter off the lead sulfate and wash free from acid with water. Dissolve the lead sulfate in ammonium acetate solution (a), make up to known volume and determine lead as lead chromate by precipitating with potassium bichromate solution. Dilute the stock solution so that 1 cc. will contain 0.1 milligram of lead.

(e) *Standard copper solution.*—Dissolve about 20 grams of copper sulfate crystals ($CuSO_4 . 5 H_2O$) in water, add 1 cc. concentrated sulfuric acid and dilute to 500 cc. Determine the copper in 50 cc. of this solution as copper oxide (CuO) by precipitation with potassium hydroxide solution. Dilute the stock solution so that 1 cc. contains 0.1 milligram of copper.

(f) *Standard zinc solution.*—Dissolve C. P. zinc in hydrochloric acid and dilute so that 1 cc. contains 0.1 mg. of zinc.

(g) *50% citric acid solution.*

(h) *2% ammonium thiocyanate solution.*

#### DETERMINATION.

(See also modified procedure.)

Acidify with hydrochloric acid from ½ to 2 liters of the sample. Concentrate in a porcelain casserole by heating slowly over the open flame to a volume of about 75 cc. Add sufficient ammonium chloride (about 2.0 grams) to hold magnesium in solution and assist in separation of sulfides. Add about 1 cc. excess of ammonia and saturate with hydrogen sulfide. Cover dish and let stand about 2 hours, add more ammonia and hydrogen sulfide, boil a few minutes, let precipitate settle, filter and wash precipitate once with hot water. The precipitate will contain all the iron, lead, copper

[1] Report Mass. State Board of Health, 1898, 582–3.
[2] *J. Ind. Eng. Chem.*, 1921, 13: 696.

and zinc, and the coloring matter will be in the filtrate. Place filter and precipitate in a small porcelain casserole; add 50 cc. of dilute nitric acid (1 to 5) and boil. Filter and wash free from acid, cool filtrate and add 5 cc. of dilute (1 to 1) sulfuric acid, concentrate carefully by boiling and heat until copious fumes of sulfuric acid are given off. Transfer to a beaker with the aid of water, add an equal volume of alcohol (95%), let stand overnight, filter off the lead sulfate and wash with dilute alcohol (50%) until free from iron. Collect the filtrate which contains iron, copper and zinc in a 250 cc. beaker.

### LEAD.

Boil the filter containing the lead sulfate with about 40 cc. of the ammonium acetate solution (a), in a small porcelain casserole, filter and wash once or twice with hot dilute ammonium acetate solution (b) and twice with water. Make filtrate up to definite volume. Add freshly prepared hydrogen sulfide water and a few drops of acetic acid to an aliquot portion. Compare the color obtained with a set of standards made by treating various amounts of the standard solution of lead sulfate (d), with hydrogen sulfide water.

### COPPER.

Boil the moderately acid filtrate, which contains iron, copper and zinc, to remove alcohol; adjust solution to a volume of about 200 cc. and add 1 gram of ammonium chloride. Heat to boiling, saturate with hydrogen sulfide gas, boil to remove precipitate sulfur, cover beaker, let stand about 2 hours or until supernatant liquid becomes clear, filter and wash the copper sulfide without intermission with water containing hydrogen sulfide. Collect filtrate in porcelain casserole. Dissolve the copper sulfide in hot dilute nitric acid (1 to 5), evaporate to dryness, take up in water, filter if solution is not clear, and adjust solution to a volume of 100 cc. Add to an aliquot, 2–3 drops of potassium ferrocyanide solution (c). Compare color obtained with standards containing proper amounts of standard copper solution (e), treated in the same way.

### ZINC.

Boil the acid filtrate from the copper sulfide precipitation to remove hydrogen sulfide, cool, neutralize with ammonium hydroxide and add 10 cc. of citric acid solution (g). Heat to boiling and if no calcium citrate separates, add small quantities of calcium carbonate until a precipitate of about 1 gram of calcium citrate is formed. Pass hydrogen sulfide through the solution until it is cool. Let stand several hours, part of the time on water bath, until supernatant liquid is clear. Filter, wash with ammonium thiocyanate solution (h), and dissolve precipitate on the filter with hot dilute hydrochloric acid. If filtrate is reddish in color, reprecipitate the zinc as before. Dispel turbidity of filtrate due to colloidal sulfur by boiling. When filtrate is clear and colorless, dilute an aliquot to 45 cc. in a 50 cc. Nessler jar. Add 5 cc. of potassium ferrocyanide solution (c), mix quickly and compare the turbidity with standard zinc solutions by viewing longitudinally the jars held over a sheet of fine print. Prepare the standards by mixing definite volumes of standard zinc solution (f), 3 cc. of concentrated hydrochloric acid, water to make 45 cc. and 5 cc. of potassium ferrocyanide solution (c). The unknown solution should contain a volume of concentrated acid equivalent to that in the standards. Do not use zinc borosilicate glassware in this determination.

### MODIFIED PROCEDURES.

(Coloring matter absent; iron, lead and zinc present.)

Add 5 cc. dilute (1 to 1) sulfuric acid to the sample, evaporate nearly to dryness and heat until copious fumes of sulfuric acid are given off. Filter off the lead sulfate and follow detailed procedure.

*Procedure for lead only.*

(Coloring matter present, iron present to extent of 1.0 mg. or less in the quantity of sample taken for analysis, and copper and zinc absent.)

After heating until fumes of sulfuric acid are given off, in the regular procedure, transfer to a beaker with the aid of water. Add 25–40 cc. of ammonium acetate solution (a), heat to boiling and precipitate the iron with ammonia. Filter, wash with dilute ammonium acetate solution (b), and water. Acidify filtrate slightly with acetic acid and determine lead colorimetrically in the filtrate by the addition of hydrogen sulfide water.

*Procedure for lead only.*

(Coloring matter, iron, zinc and copper absent.)

Add 5 cc. of concentrated sulfuric acid to the sample, evaporate nearly to dryness and heat until copious fumes of sulfuric acid are given off. Transfer to a beaker with the aid of water, add 25–40 cc. of ammonium acetate solution (a), and determine lead colorimetrically by the addition of hydrogen sulfide water.

It will be recommended that the above methods be given careful study next year.

In 1919, the association extended the methods of water analysis to cover allied products, such as brine and salt. The standard for salt which has been promulgated in connection with the enforcement of the Federal Food and Drugs Act provides that table salt (dairy salt) shall contain on a water free basis, not more than 1.4 per cent of calcium sulfate, 0.5 per cent of calcium and magnesium chlorides and 0.1 per cent of matters insoluble in water. It is important, when the purity of shipments of salt is questioned, that the methods of analysis be uniform and specific. The referee, at this time, is in a position to submit only methods for determination of moisture, matters insoluble in water and matters insoluble in acid. They are as follows:

## SALT.

The samples should be representative. If the shipment is packed in bags or other containers, collect one sample from each of five containers.

### MOISTURE.

#### DETERMINATION.

Place 10 grams of the well-mixed sample in a weighed Erlenmeyer flask, capacity 200 cc. Insert small funnel in neck. Ignite to constant weight over low flame of gas stove. Call the loss moisture and express in per cent.

### MATTERS INSOLUBLE IN WATER.

#### DETERMINATION.

Treat 10 grams of the well-mixed sample with 200 cc. of water and let stand 30 minutes, stirring frequently. Filter through Gooch crucible with mat, dried at 110°C. Wash residue free from chloride. Dry to constant weight at 110°C. Express results in per cent. If residue exceeds 0.1% determine its nature.

### MATTERS INSOLUBLE IN ACID.

#### DETERMINATION.

Treat 10 grams of the well-mixed sample with 190 cc. of water and 10 cc. of concentrated hydrochloric acid, boil 2–3 minutes, let stand 30 minutes, stirring frequently. Filter through Gooch crucible with mat, dried at 110° C. Express results in per cent.

Methods of combining radicals are not uniform among water analysts. While there is a wide diversity of opinion as to the advisability of expressing water analyses in the form of salts, the fact remains that in the administration of Federal and State laws it is usually necessary to present to physicians and to the court water analyses expressed in the form of salts. For this reason, the tentative method[1] of reporting results has been extended for the benefit of the analyst who does not continually make analyses of water. The principles involved in the tentative methods adopted by the association have been retained. The extended method follows in detail:

## METHOD OF REPORTING RESULTS.

Report radicals and anhydrous salts in terms of milligrams per liter or, in the case of highly concentrated waters, in terms of grams per liter. For the benefit of physicians, in the case of medicinal waters, report also the salts in terms of grains per quart, using the factor 0.014604 to convert milligrams per liter to grains per quart. In reporting salts in terms of grains per quart, convert those salts which have water of crystallization to the hydrated form as expressed in the U. S. Pharmacopoeia and National Formulary, and convert the bicarbonates of magnesium and of calcium to equivalent amounts of the respective carbonates. Use the following factors in these calculations:

From $Na_2SO_4$ to $Na_2SO_4 . 10\ H_2O = 2.2682$;
$MgSO_4$ to $MgSO_4 . 7\ H_2O = 2.0476$;
$CaSO_4$ to $CaSO_4 . 2\ H_2O = 1.2647$;
$Mg(HCO_3)_2$ to $MgCO_3 = 0.5762$;
$Ca(HCO_3)_2$ to $CaCO_3 = 0.6174$.

Calculate other less used factors and employ them when necessary.

In special cases, as in the analysis of drainage waters, boiler waters, etc., do not report the salts in grains per quart but report instead the reacting values of the radicals. When a complete analysis is made report the error of analysis and state how it is distributed. Report only significant figures.

Report iron and aluminium together when present in unimportant quantities and in calculations consider them as iron. When iron and aluminium are present in larger quantities, make the separation and report each separately.

In calculating the hypothetical combinations of acid and basic ions, join nitrous, nitric, metaboric and arsenic acids to sodium; iodine and bromine to potassium; and phosphoric acid to calcium. Assign the residual basic ions in the following order: Ammonium, lithium, potassium, sodium, magnesium calcium, strontium, manganese, iron and aluminium—to the residual acid ions in the following order: Chlorine, sulfuric acid ion, carbonic acid ion and bicarbonic acid ion. In case the bicarbonic acid ion is not present in a sufficient quantity to join with all the calcium, the residual calcium is joined to silica to form calcium silicate, and manganese iron and aluminium are calculated to the oxids $Mn_3O_4$, $Fe_2O_3$ and $Al_2O_3$, respectively.

[1] *Assoc. Official Agr. Chemists, Methods*, 1920, 21–41.

Use equivalent combining weights or their reciprocals in uniting the radicals, and when necessary for the purpose of comparison, in reducing salts to radicals and reuniting the radicals in the order specified on page 385.

TABLE 3.

*Equivalent combining weights and their reciprocals based on international atomic weights, 1921.*

| NEGATIVE RADICALS | EQUIVALENT COMBINING WEIGHTS | RECIPROCALS OF EQUIVALENT COMBINING WEIGHTS | NEGATIVE RADICALS | EQUIVALENT COMBINING WEIGHTS | RECIPROCALS OF EQUIVALENT COMBINING WEIGHTS |
|---|---|---|---|---|---|
| $NO_2$ | 46.008 | .0217 | $SiO_2$ | 30.15 | .0332 |
| $NO_3$ | 62.008 | .01613 | $SiO_3$ | 38.15 | .0262 |
| $BO_2$ | 42.9 | .02331 | O | 8.0 | .1250 |
| $AsO_4$ | 46.32 | .0216 | F | 19.0 | .0526 |
| I | 126.92 | .0079 | Cl | 35.46 | .028201 |
| Br | 79.92 | .01251 | $SO_4$ | 48.03 | .020820 |
| $PO_4$ | 31.68 | .03156 | $CO_2$ | 22.0025 | .0454 |
| HS | 33.068 | .0302 | $CO_3$ | 30.0025 | .0333 |
| S | 16.03 | .0624 | $HCO_3$ | 61.013 | .016390 |
| ..... | ..... | ..... | ..... | ..... | ..... |
| Positive radicals | | | Positive radicals | | |
| $NH_4$ | 18.04 | .0554 | Sr | 43.815 | .0228 |
| Li | 6.94 | .1441 | Ba | 68.685 | .0145 |
| K | 39.10 | .02557 | Mn | 27.465 | .0364 |
| Na | 23.00 | .043478 | Fe'' | 27.92 | .035817 |
| Mg | 12.16 | .082237 | Fe''' | 18.6133 | .053725 |
| Ca | 20.035 | .049913 | Al | 9.0333 | .110702 |

| SALTS | EQUIVALENT COMBINING WEIGHTS | RECIPROCALS OF EQUIVALENT COMBINING WEIGHTS | SALTS | EQUIVALENT COMBINING WEIGHTS | RECIPROCALS OF EQUIVALENT COMBINING WEIGHTS |
|---|---|---|---|---|---|
| $NH_4Cl$ | 53.50 | .0187 | $MgCl_2$ | 47.62 | .021000 |
| LiCl | 42.40 | .0236 | $MgSO_4$ | 60.19 | .016614 |
| $Li_2SO_4$ | 54.97 | .0182 | $MgCO_3$ | 42.1625 | .02372 |
| $Li_2CO_3$ | 36.9425 | .0271 | $Mg(HCO_3)_2$ | 73.173 | .01367 |
| $LiHCO_3$ | 67.953 | .0147 | $Mg(NO_3)_2$ | 74.168 | .01348 |
| KCl | 74.56 | .0134 | $CaCl_2$ | 55.495 | .018020 |
| $K_2SO_4$ | 87.13 | .01148 | $CaSO_4$ | 68.065 | .014692 |
| $K_2CO_3$ | 69.1025 | .01447 | $CaCO_3$ | 50.0375 | .0200 |
| $KHCO_3$ | 100.113 | .00999 | $Ca(HCO_3)_2$ | 81.048 | .01234 |
| KI | 166.02 | .0060 | $CaSiO_3$ | 58.185 | .0172 |
| K Br | 119.02 | .00840 | $Ca_3(PO)_2$ | 51.715 | .0193 |
| NaCl | 58.46 | .0171 | $SrSO_4$ | 91.845 | .0111 |
| Na Br | 102.92 | .0097 | $SrCO_3$ | 73.8175 | .0135 |
| NaI | 149.92 | .0067 | $Sr(HCO_3)_2$ | 104.828 | .0095 |
| $Na_2SO_4$ | 71.03 | .01407 | $BaSO_4$ | 116.715 | .0086 |
| $Na_2CO_3$ | 53.0025 | .0189 | $Ba(HCO_3)_2$ | 129.698 | .0077 |
| $NaHCO_3$ | 84.013 | .01190 | $MnSO_4$ | 75.495 | .0132 |
| $NaNO_2$ | 69.008 | .0145 | $MnCO_3$ | 57.4675 | .0174 |
| $NaNO_3$ | 85.008 | .01176 | $Mn(HCO_3)_2$ | 88.478 | .0113 |
| $NaBO_2$ | 65.9 | .01517 | $FeSO_4$ | 75.95 | .013166 |
| $Na_3AsO_4$ | 69.32 | .0144 | $Fe_2(SO_4)_3$ | 66.6433 | .015005 |
| NaF | 42.00 | .0238 | $Fe_2CO_3$ | 57.22833 | .0175 |
| NaHS | 56.068 | .0178 | $Fe(HCO_3)_2$ | 88.933 | .01124 |
| $Na_2S$ | 39.03 | .0256 | $Fe_2O_3$ | 26.613 | .0376 |
| $Na_2SiO_3$ | 61.15 | .0163 | $Al_2(SO_4)_3$ | 57.063 | .017524 |
| ..... | ..... | ..... | $Al_2O_3$ | 17.033 | .0587 |

The equivalent combining weight of a radical is obtained by dividing its weight by its valence. The equivalent combining weight of a salt is obtained by dividing its molecular weight by the product of the valency of the basic element and the number of atoms of the basic element in the salt. The equivalent weights and their reciprocals (reaction coefficients) for the radicals and salts ordinarily used are set forth in Table 3.

The procedure in calculating the hypothetical combinations by the use of the equivalent combining weights and their reciprocals is as follows:

Multiply the weights obtained, expressed in milligrams per liter, or, in the case of highly concentrated waters, in grams per liter, for each radical to be combined, by the corresponding reciprocal of the equivalent combining weights. If the sodium and potassium are to be determined by calculation, as is frequently the case, subtract the sum of the values obtained (reacting values) for the basic radicals from the sum of the reacting values for the acid radicals. The difference represents the reacting value of the undetermined sodium and potassium. When all the constituents in the water have been determined the sums of the reacting values of the acid and of the basic radicals should be very nearly the same. In this case, if the difference is reasonable and well within the limit of accuracy of the methods employed, it may be distributed equally among all the radicals determined, or among those which the analyst believes to be less accurate than the others. If the difference is unreasonably great, repeat the analysis in whole or in part. The sums of the reacting values of the acid and basic radicals must be equal before proceeding with the calculation. Obtain the reacting values of the salts by subtracting in succession the reacting values of the radicals in the specified order. For example, the reacting values for the radicals in a concentrated water are as follows: Cl, 21.573; $SO_4$, 4237.934; $CO_3$, 18.498; $HCO_3$, 20.487; Na, 528.231; Mg, 3747.451 and Ca, 22.810. Then 528.231 − 21.573 (reacting value of Cl and of NaCl) = 506.658 (remaining reacting value of Na); 4237.934 − 506.658 (reacting value of $Na_2SO_4$) = 3731.276 (remaining reacting value of $SO_4$); 3747.451 − 3731.276 (reacting value of $MgSO_4$) = 16.175 (remaining reacting value of Mg); 18.498 − 16.175 (reacting value of $MgCO_3$) = 2.323 (remaining reacting value of $CO_3$); 22.810 − 2.323 (reacting value of $CaCO_3$) = 20.487 (remaining reacting value of Ca and also the reacting value of $HCO_3$ and of Ca $(HCO_3)_2$). The following reacting values for the salts are thus obtained: NaCl, 21.573; $Na_2SO_4$, 506.658; $MgSO_4$, 3731.276; $MgCO_3$, 16.175; $CaCO_3$, 2.323; Ca $(HCO_3)_2$, 20.487. To convert these figures to milligrams per liter of the respective salts divide each of them by the reciprocal of the equivalent combining weights of the salt in question or preferably multiply each of them by the equivalent combining weight of the respective salt.

It is recommended—

(1) That the method for the determination of iodine in the presence of chlorine and bromine, page 381, be adopted as a tentative method. The method has not been published in the Proceedings as provided by By-law No. 7.

(2) That the method for the determination in salt of moisture, matters insoluble in water and matters insoluble in acid, page 384, be adopted as a tentative method.

(3) That the method of reporting results of water analyses, page 385, be adopted as a tentative method.

(4) That the tentative method of reporting results of water analyses[1] be dropped.

(5) That the quantitative methods for the determination of small quantities of copper and zinc in waters, page 382, be studied during the next year.

## REPORT ON TANNING MATERIALS AND LEATHER.

By F. P. VEITCH (Bureau of Chemistry, Washington, D. C.), *Referee.*

Work of the referee continues to be materially hampered by the fact that so few members of this association are directly interested in the subject of analysis of tanning materials and leather. It is believed, however, that despite this handicap the association should continue work of this character because as leather-making materials become scarcer greater interest will be taken. Furthermore, the methods of this association should apply to all of those materials which are of immediate and direct interest to the farm population.

In view of this lack of active interest, to save time and avoid unnecessary printing, the referee will refrain from giving the details of the methods employed. This report will be confined to a general statement of the various problems which have been studied, to the conclusions reached and to recommendations for further work. Those who may be interested in the details of the methods which have been studied in connection with the analysis of tanning materials and leather are referred to the Journal of the American Leather Chemists Association and to the Journal of the Society of Leather Trades Chemists for the past five years.

### ANALYSIS OF LEATHER.

#### EFFECT OF ATMOSPHERIC HUMIDITY ON DETERMINATION OF MOISTURE.

During the past year the referee and several collaborators continued the work on the effect of atmospheric humidity and temperature on the determination of moisture in leather and tanning materials. It was found that the atmospheric humidity existing at the time of the determination of moisture in the sample may affect the moisture result as much as ½ per cent, and it is believed that this is true of practically all organic materials. Though the effect is observable in the vacuum oven, it is not quite as marked as when the drying is done in the ordinary hot-water oven.

It was observed also that in drying leather over very long periods at the same atmospheric humidity there is a constant, but very small, loss in weight, the nature of which was not determined.

[1] *Assoc. Official Agr. Chemists, Methods*, 1920, 38.

Work on this subject will be continued during the coming year, and efforts will be made to secure more extended cooperation within this association.

### SOLVENTS FOR EXTRACTION OF GREASES.

Some work was done to determine the proper solvent to use in extracting grease, oils and soaps from leather. Petroleum ether does not extract all of the waxes, the oxidation products of oils and fats or the soap which may have been added to the leather in currying. No solvent has been found which will take these out without removing tannins or other constituents which should be retained.

A number of investigators, including Wilson and Kern, Levi and Orthmann, and committees of the American Leather Chemists Association and of the Society of Leather Trades Chemists of England, have worked extensively along this line. Their complete papers or abstracts are to be found in the Journal of the American Leather Chemists Association, 1918 and since.

After a full consideration of previous work, the referee decided to confine investigations to the effect of ethyl ether and chloroform as compared with petroleum ether. Without going into the analytical details, which are to be found in the Journal of the American Leather Chemists Association, it may be said: (*a*) That chloroform is the best solvent so far found for greases, waxes and oxidation products in leather; (*b*) that dry Ivory soap alone is practically insoluble in chloroform and petroleum ether; (*c*) that the presence of moisture in leather will increase the quantity of soap fats extracted by chloroform and by petroleum ether, but the effect of the quantity of moisture usually present in well air-dried leather is small; (*d*) that the presence of tannic acid in leather, probably by decomposing the soap and setting free the fatty acids, increases the solubility of soap and fats very greatly but mostly in chloroform; (*e*) that the grease extracts from a material containing uncombined tannic acid contains small quantities of tannic acid or related bodies and loses weight indefinitely in drying; (*f*) that magnesium salts added to leather will lead to the extraction of the soap as a whole if opportunity is given for the formation of magnesium soaps since the latter are very soluble both in chloroform and petroleum ether; (*g*) that calcium soaps are extracted by chloroform in a colloidal solution but are practically insoluble in petroleum ether; and (*h*) that while chloroform is a much better solvent for the fats of soap added to the leather than is petroleum ether, all the soap fats are probably not removed by chloroform when the extraction is made as usual. Since soaps are often used in currying leather, any undecomposed particles which may remain in the leather will probably be removed in the water extraction of the leather and the results on fat, if it has not previously been removed with the solvent,

are too low. More work needs to be done on the solubility of soaps from different greases before it will be known how serious this error may be.

### DETERMINATION OF TANNIN.

That the methods now used for the determination of tannin in tanning materials do not give accurate results but indicate a higher content of tannin than the material actually contains, has long been known. In this connection the present referee, in a paper, "A Discussion of Methods for the Estimation of Tannin"[1], read before the annual meeting of this association in 1904, stated:

> The method known as the hide-powder method, while not formally adopted as official by this association, is used exclusively for the estimation of tannin in tanning materials. It is well recognized, however, that the method yields inaccurate results. The chief sources of error appear to be a continuous absorption of solids from the solution by the filter paper during the preceding determination of soluble solids, and an undoubtedly greater error due to the absorption of coloring matter and other nontannin matters by the hide powder. The first may produce a minus error, the latter produces a plus error. Experimental demonstration is hardly needed of the fact that hide powder removes other constituents than tannin from tannin solutions brought in contact with it, as such absorption, occlusion, etc., is a common property of all precipitates or other solid matter in contact with solutions of solids, and prolonged washing is frequently required to free them from materials held in this way. Nevertheless we have experiments showing that in addition to tannin most of the nontannin constituents common to solutions of tanning materials are absorbed to a serious extent by hide powder, as may be seen from the work of Proctor and Blockey[2], who found the absorption of gallic acid, quintol, catechol, and catechin when present in solution with gallotannic acid or quebracho tannin was from 44 per cent to 106 per cent of the amount present. Dextrin and glucose were absorbed in very much smaller quantities or lowered the absorption of the tannin.
>
> A lively appreciation of these facts, together with the time and work required to obtain results, has greatly stimulated the search for more accurate and quicker methods. The methods and proposals which have appeared as a result of this activity are of such a character that, while I cannot offer anything better than the hide-powder method, a few words of criticism and a brief statement of what must be avoided would seem to be timely, and may be of service to many who at first sight are favorably impressed with some of these methods.

Although many varied procedures have been proposed, the indirect method of determining tannin by difference through removal with a standard hide powder remains preferred, and, indeed, the only recognized procedure, simply because a better one is not known. The chemists interested in tanning and leather have sought in vain a direct method for estimating tannin, unless the procedure which has been studied more recently by Wilson and Kern[3] should yield a satisfactory method.

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[1] U. S. Bur. Chem. Bull. **90**: 215.
[2] *J. Soc. Chem. Ind.*, 1903, **22**: 482.
[3] *J. Am. Leather Chem. Assoc.*, 1920, **15**: 295.

This procedure is now under investigation by the referee with a view to determining, more definitely than has so far been done, its reliability and accuracy. In this work the referee hopes to secure the cooperation of the other members of this association during the coming year.

The problem is complicated materially by the absorption of catechin as shown by Proctor and Blockey[1]. These investigators make the following statement concerning catechin:

> Experiments were also made with catechin, which stands in a different relation from the other non-tanning substances to tannins, since the catechins are not tannin derivatives, but rather the root substances from which the catechol tannins themselves are derived by abstraction of water. It will be seen that the catechin experimented with was practically wholly absorbed by the hide-powder in the filter method, but this can hardly be considered an error, since it is probable that during the tanning process the catechin becomes gradually dehydrated and converted into an actual tannin. The specimen used was obtained as pure from Merck, and was white and crystalline, but showed itself more soluble in cold water than is usually stated to be the case. A saturated solution in cold water showed decided tanning properties, producing an undertanned yellowish leather. These tanning properties were distinctly increased by boiling the solution for some hours before use, and both the solution itself and the leather produced became decidedly redder in colour. One of us proposes to investigate this point more completely, as it is of considerable practical importance in view of the large quantity of catechin contained in gambier and cutch.

These observations have been confirmed recently by Wilson and Kern[2], and the fact that such changes take place during the analytical processes may introduce errors in the process itself, or, if they take place, as they probably do, in the tan yard, may leave in question the procedure which may be employed in the determination of so-called tannin.

## RECOMMENDATIONS.

It is recommended—

(1) That work be continued on the solubility of various soaps in different solvents and upon a method, probably first breaking up the soap by heating the leather with an acid, for the extraction of total soaps in leather.

(2) That investigations of a direct method for the determination of tannin in tanning materials be continued.

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[1] *J. Soc. Chem. Ind.*, 1903, **22**: 482.
[2] *J. Am. Leather Chem. Assoc.*, 1920, **15**: 295.

# REPORT ON INSECTICIDES AND FUNGICIDES.

By J. J. T. GRAHAM (Bureau of Chemistry, Washington, D. C.), *Referee.*

The cooperative work on insecticides and fungicides for 1921 included a study of methods for the determination of total arsenic, arsenious oxide and calcium oxide in calcium arsenate; for the determination of arsenious oxide and zinc oxide in zinc arsenite; and for the determination of lead oxide, zinc oxide and copper in a mixture of Bordeaux, lead arsenate and zinc arsenite. Methods for the determination of arsenious oxide in Paris green, total arsenic in London purple, and magnesium oxide in magnesium arsenate were also considered by the referee.

Reports were received from seven analysts in three laboratories.

The following methods were tested:

## ZINC ARSENITE.

The zinc arsenite sent to the collaborators was a commercial sample from a well-known insecticide manufacturer.

### ZINC OXIDE.

*Mercury-Thiocyanate Method*[1].

#### REAGENT.

Dissolve 27.0 grams of mercuric chloride and 38 grams of potassium thiocyanate in 1 liter of water. In lieu of the potassium thiocyanate, 30 grams of ammonium thiocyanate may be used.

#### DETERMINATION.

Weigh 2.0 grams of the sample and transfer to a beaker. Dissolve in 80 cc. of hydrochloric acid (1 to 3), wash into a 200 cc. volumetric flask, and dilute to volume. Thoroughly mix the solution and filter through a dry filter. Transfer a 25 cc. aliquot to a beaker and add 5 cc. of concentrated hydrochloric acid. If there is much iron present, reduce it at this point by adding a little sodium bisulfite and heating on the steam bath until the odor of sulfur dioxide has largely disappeared. Cool, dilute to about 100 cc. and add 35–40 cc. of the mercury-thiocyanate reagent with vigorous stirring. Allow to stand at least an hour with occasional stirring. Filter through a tared Gooch crucible, wash with water containing 20 cc. of the mercury-thiocyanate reagent per liter, and dry to constant weight at 105°C. From this weight calculate the per cent of zinc oxide in the sample, using the factor 0.16331.

### ARSENIOUS OXIDE.

#### REAGENTS.

(a) *Starch indicator.*—Prepare as directed under Paris green[1].

(b) *Standard arsenious oxide solution.*—Prepare as directed under Paris green[1].

(c) *Standard iodine solution.*—Prepare as directed under Paris green[2].

(d) *Standard bromate solution.*—Dissolve 1.688 grams of pure potassium bromate or 1.525 grams of pure sodium bromate in water and dilute to 1 liter. One cc. of this

[1] *Trans. Am. Inst. Met.*, 1914, **8**: 146; *J. Am. Chem. Soc.*, 1918, **40**: 1036.
[2] *Assoc. Official Agr. Chemists, Methods*, 1920, 53.

solution is approximately equal to 0.00300 gram of arsenious oxide. To standardize, transfer 25 cc. aliquots of the standard arsenious oxide solution to 500 cc. Erlenmeyer flasks, add 15 cc. of concentrated hydrochloric acid, dilute to 100 cc., heat to 90°C. and titrate with the bromate solution, using 5 drops of a 0.2% solution of methyl orange as indicator. The indicator should not be added until near the end of the titration, and the liquid should be agitated continuously in order to avoid local excess of the bromate solution. The bromate should be added very slowly when approaching the end of the titration, the end point being shown by a change from red to colorless.

TABLE 1.

*Cooperative results on zinc arsenite.*

| ANALYST | ZINC OXIDE | ARSENIOUS OXIDE. | | |
|---|---|---|---|---|
| | | BROMATE METHOD | | HEDGES METHOD |
| | | Hot | Cold | |
| | *per cent* | *per cent* | *per cent* | *per cent* |
| L. N. Markovitz, Bureau of Chemistry, Washington, D.C. | 56.40 | 41.16 | 41.18 | 41.28 |
| | 56.40 | 41.22 | 41.18 | 41.30 |
| Average | 56.40 | 41.19 | 41.18 | 41.29 |
| H. L. Fulmer, Guelph, Canada. | 56.46 | ..... | ..... | ..... |
| | 56.39 | ..... | ..... | ..... |
| Average | 56.43 | | | |
| J. J. T. Graham. | 56.80 | 41.06 | 41.12 | 40.99 |
| | 56.73 | 41.06 | 41.12 | 40.94 |
| | 56.87 | 41.12 | 41.12 | 40.99 |
| Average | 56.80 | 41.08 | 41.12 | 40.97 |
| Percy O'Meara, E. Lansing, Mich. | 56.44 | 41.16 | 41.20 | 41.50 |
| | 56.56 | 41.08 | 41.16 | 41.80 |
| | ..... | ..... | ..... | 42.10 |
| Average | 56.50 | 41.12 | 41.18 | 41.80 |
| C. M. Smith, Bureau of Chemistry, Washington, D. C. | 56.59 | 41.08 | 41.08 | ..... |
| | 56.51 | 41.08 | 41.08 | ..... |
| | 56.59 | 41.14 | 41.14 | ..... |
| | 56.45 | 41.14 | 41.14 | ..... |
| | 56.46 | 41.08 | 41.08 | ..... |
| Average | 56.52 | 41.10 | 41.10 | |
| F. L. Hart, Bureau of Chemistry, Washington, D. C. | ..... | 41.26 | 41.20 | 41.26 |
| | ..... | 41.22 | 41.16 | 41.22 |
| | ..... | 41.29 | 41.16 | ..... |
| Average | | 41.26 | 41.17 | 41.24 |
| General Average | 56.55 | 41.14 | 41.13 | 41.34 |

DETERMINATIONS.

*Bromate Method.*—(1) Transfer a 25 cc. aliquot of the solution prepared for the determination of zinc to a 500 cc. Erlenmeyer flask, add 20 cc. of concentrated hydrochloric acid and dilute to 100 cc. Heat to 90°C. and titrate with the standard bromate solution.

(2) Proceed as in (1) without heating the solution.

*Hedges Method.*

Proceed as directed under Paris green[1].

## DISCUSSION.

The results for zinc oxide agree very well. The method is easy to manipulate and is much preferable to other methods now in use for the determination of zinc. The results for arsenious oxide by the bromate method give very close checks, the maximum variation of all the results being 0.23%. They show that with careful work the method is accurate whether carried out at ordinary room temperature or at 90°C., although the end point is a little sharper at the latter temperature. This titration, influenced by the presence of nitrates in the sample, will be discussed more fully under calcium arsenate.

## CALCIUM ARSENATE.

The sample used was prepared from commercial materials, by pouring a solution of arsenic and arsenious acids into milk of lime, with vigorous stirring. After standing for some time, the mixture was filtered, dried, passed through a 40 mesh sieve and thoroughly mixed. The arsenious oxide was added in order to test the method for arsenious oxide.

### TOTAL ARSENIC.

REAGENTS.

(a) *Starch indicator.*—Prepare as directed under Paris green[2].

(b) *Standard arsenious oxide solution.*—Prepare as directed under Paris green[2]. To convert arsenious oxide to arsenic oxide use the factor 1.16168.

(c) *Standard iodine solution.*—Prepare as directed under Paris green[2].

(d) *Standard bromate solution.*—Prepare as directed under zinc arsenite, page 392.

DETERMINATION.

*Official Distillation Method.*

Proceed as directed under Paris green[3], using an amount of the sample equal to the arsenic oxide equivalent of 500 cc. of the standard iodine solution and titrating 200 cc. of the distillate. The number of cc. of standard iodine solution used represents directly the total per cent of arsenic in the sample expressed as arsenic oxide.

*Bromate Method*[4].

Proceed as directed under the official distillation method until the distillate is made to volume in a liter graduated flask, using an amount of the sample equal to the arsenic oxide equivalent of 500 cc. of the standard bromate solution. Transfer 200 cc. aliquots of the distillate to 500 cc. Erlenmeyer flasks, heat to 90°C. and titrate with the

[1] *Assoc. Official Agr. Chemists, Methods*, 1920, 55.
[2] *Ibid.*, 53.
[3] *Ibid.*, 54.
[4] *Z. anal. Chem.*, 1893, 32: 415; *J. prakt. Chem.*, 1915, 91: 133.

standard bromate solution, using 5 drops of a 0.2% solution of methyl orange as indicator. The indicator should not be added until near the end of the titration, and the solution should be agitated continuously in order to avoid local excess of the bromate solution. The number of cc. of standard bromate solution used represents directly the total per cent of arsenic in the sample expressed as arsenic oxide.

*Modified Gooch and Browning Method.*

Weigh an amount of the sample equal to the arsenic oxide equivalent of 100 cc. of the standard iodine solution, transfer to a 500 cc. Erlenmeyer flask; add 5 cc. of concentrated sulfuric acid, dilute to 150–200 cc. and add 1 gram of potassium iodide. Boil until the volume is reduced to about 40 cc. A glass boiling tube will prevent superheating and loss of the solution by violent boiling. Cool, dilute to 150–200 cc., remove the excess iodine by titration with 0.05N sodium thiosulfate, nearly neutralize the sulfuric acid with a solution of sodium hydroxide (40 grams in 100 cc. of water) and finish the neutralization with sodium bicarbonate, adding 4–5 grams in excess and titrate with standard iodine using starch solution as indicator. The number of cc. of standard iodine solution used represents directly the total per cent of arsenic in the sample expressed as arsenic oxide.

NOTE.—The boiling tube mentioned above is made from a piece of glass tubing of about 2 or 3 mm. internal diameter, by sealing it about 1.5 cm. from one end. During boiling this end is placed in the solution.

## ARSENIOUS OXIDE.

### REAGENTS.

The reagents used are described under total arsenic, page 394.

### DETERMINATION.

*Bromate Method.*

(Not applicable in presence of nitrates.)

(1) Weigh an amount of the sample equal to the arsenious oxide equivalent of 300 cc. of the standard bromate solution. Transfer to a 500 cc. Erlenmeyer flask and dissolve in 100 cc. of hydrochloric acid (1 to 3). Heat to 90°C. and titrate with the standard bromate solution, using 5 drops of a 0.2% methyl orange solution as indicator. The number of cc. of bromate solution used, divided by 3, gives the per cent of arsenious oxide in the sample.

(Applicable in presence of small amounts of nitrates.)

(2) Proceed as in (1) except that the titration is made at room temperature.

## CALCIUM OXIDE.

### DETERMINATION.

Method 1.—Dissolve 2.0 grams of the sample in 80 cc. of acetic acid (1 to 3), transfer to a 200 cc. volumetric flask and make to volume. Filter through a dry filter and transfer a 50 cc. aliquot to a beaker; dilute to 200 cc., heat to boiling and precipitate the calcium with ammonium oxalate solution. Allow the beaker to stand for 3 hours on the steam bath, filter and wash with hot water. Dissolve the precipitate in 200 cc. of water containing 25 cc. of dilute sulfuric acid (1 to 4), heat to about 70°C. and titrate with standard potassium permanganate solution.

(Not applicable to calcium arsenate containing lead.)

Method 2.—Weigh 2.0 grams of the sample, transfer to a beaker, add 5 cc. of hydrobromic acid (sp. gr. 1.31), and 15 cc. of hydrochloric acid (sp. gr. 1.19), and evaporate to dryness under a hood to remove arsenic; repeat the treatment; add 20 cc. of hydrochloric acid and again evaporate to dryness. Take up with water and a little hydrochloric acid, filter into a 200 cc. volumetric flask, wash and make to volume. Trans-

TABLE 2.

*Cooperative results on calcium arsenate containing calcium arsenite.*

| ANALYST | TOTAL ARSENIC CALCULATED AS ARSENIC OXIDE | | | ARSENIOUS OXIDE | | CALCIUM OXIDE | | |
|---|---|---|---|---|---|---|---|---|
| | Official Distillation Method | Bromate Method | Modified Gooch and Browning Method | Bromate Method | | Method 1 | Method 2 | |
| | | | | Hot | Cold | | Titrated | Ignited |
| | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* |
| Percy O'Meara. | 39.96 | 39.67 | 40.65 | 4.63 | 4.70 | 29.74 | 29.54 | ..... |
| | 39.92 | 39.77 | 40.65 | 4.57 | 4.73 | 29.64 | 29.70 | ..... |
| Average..... | 39.94 | 39.72 | 40.65 | 4.60 | 4.72 | 29.69 | 29.62 | ..... |
| J. J. T. Graham. | 39.39 | 39.87 | 39.58 | 4.76 | 4.76 | 29.60 | 29.60 | 29.48 |
| | 39.54 | 39.87 | 39.58 | 4.74 | 4.76 | 29.60 | 29.60 | 29.76 |
| | 39.44 | 39.87 | 39.54 | 4.77 | 4.76 | 29.48 | 29.54 | 29.84 |
| | 39.44 | 39.81 | ..... | ..... | .... | ..... | ..... | ..... |
| Average..... | 39.45 | 39.86 | 39.57 | 4.76 | 4.76 | 29.56 | 29.58 | 29.69 |
| H. L. Fulmer. | ..... | ..... | ..... | .... | .... | 30.17 | 30.23 | ..... |
| | ..... | ..... | ..... | .... | .... | 30.02 | 30.28 | ..... |
| Average..... | ..... | ..... | ..... | .... | .... | 30.10 | 30.26 | ..... |
| F. L. Hart. | 39.55 | 39.52 | 39.82 | 4.71 | 4.76 | 29.56 | 29.42 | ..... |
| | 39.55 | 39.57 | 39.64 | 4.76 | 4.78 | 29.49 | 29.48 | ..... |
| | 39.46 | 39.63 | 39.82 | .... | .... | 29.58 | 29.48 | ..... |
| | 39.51 | 39.57 | 39.55 | .... | .... | 29.63 | 29.54 | ..... |
| | ..... | ..... | 39.69 | .... | .... | ..... | ..... | ..... |
| Average..... | 39.52 | 39.57 | 39.70 | 4.74 | 4.77 | 29.55 | 29.48 | ..... |
| L. N. Markovitz. | 39.40 | 39.69 | 39.21 | 4.73 | 4.75 | 30.02 | 29.72 | 29.98 |
| | 39.45 | 39.53 | 39.33 | 4.73 | 4.73 | 29.86 | 29.68 | 30.18 |
| | 39.67 | 39.74 | 39.28 | 4.74 | .... | 29.90 | ..... | ..... |
| | ..... | ..... | ..... | .... | .... | 29.82 | ..... | ..... |
| Average..... | 39.51 | 39.65 | 39.27 | 4.73 | 4.74 | 29.90 | 29.70 | 30.08 |
| G. E. Miller, Bureau of Chemistry, Washington, D. C. | 39.61 | 39.43 | 39.61 | .... | .... | 30.07 | 30.13 | ..... |
| | 39.61 | 39.49 | 39.69 | .... | .... | 30.28 | 29.97 | ..... |
| Average..... | 39.61 | 39.46 | 39.65 | .... | .... | 30.18 | 30.05 | ..... |
| General average | 39.57 | 39.67 | 39.71 | 4.71 | 4.75 | 29.79 | 29.73 | 29.85 |

fer a 50 cc. aliquot to a beaker, add 10 cc. of hydrochloric acid and a few drops of nitric acid; boil and make slightly alkaline with ammonia. Let it stand a few minutes and filter. Dissolve the precipitate in a little hydrochloric acid, reprecipitate, filter through the same paper and wash with hot water. To the combined filtrates and washings add 20 cc. of acetic acid (1 to 3) and adjust the volume to about 200 cc. Heat to boiling, precipitate with ammonium oxalate solution and allow to stand for 3 hours on a steam bath. Filter and wash with hot water. Ignite and weigh as calcium oxide; or dissolve the precipitate in 200 cc. of water containing 25 cc. of dilute sulfuric acid (1 to 4), heat to about 70°C. and titrate with standard potassium permanganate.

## DISCUSSION.

The results of the work on calcium arsenate are somewhat at variance, and it is to be regretted that so few reports have been received. The results for total arsenic by the bromate method agree very well with the official distillation method and are more uniform than by the modified Gooch and Browning method. The largest variations from the mean are found in the results by the modified Gooch and Browning method. It is recommended that no further work be done on this method.

The results for arsenious oxide by the bromate method are very good and show that with careful work the accuracy of the method is not affected by the temperature of the solution, between the limits of room temperature and 90°C.

It is impossible to make this titration in hot solution in the presence of an appreciable amount of nitrates as the methyl orange is bleached, thus obscuring the end point. This is not the case, however, when the titration is made at room temperature. The referee has found that the addition of 0.25 gram of lead nitrate to the titration flask makes no difference in the results when titrated immediately in the cold, but when heated the indicator is instantly bleached without the addition of any of the bromate solution.

The results reported by each analyst for calcium oxide agree very well by the two methods, but there is some variation among the different analysts. The results of cooperative tests last year were more nearly uniform, and the referee believes that both of these methods are worthy of adoption by the association.

Commercial calcium arsenates frequently contain a small amount of lead arsenate, and in the analysis of such a mixture by Method 2 it is necessary to remove the lead before precipitating the calcium or the precipitate will be contaminated with lead oxalate. This is not the case, however, with Method 1, as the lead arsenate is insoluble in the acetic acid and is separated from the calcium in the preliminary treatment.

### GENERAL PROCEDURE FOR THE ANALYSIS OF A PRODUCT CONTAINING ARSENIC, ANTIMONY, LEAD, COPPER, ZINC, IRON, CALCIUM, MAGNESIUM, ETC.

(Applicable to such preparations as Bordeaux-lead arsenate, Bordeaux-zinc arsenite, Bordeaux-Paris green, Bordeaux-calcium arsenate, etc.)

A sample was prepared by mixing thoroughly 200 grams of Bordeaux mixture, made from commercial copper sulfate and lime, with 400 grams of commercial lead arsenate and 200 grams of commercial zinc arsenite. Each material had been passed through a 40 mesh sieve, thoroughly mixed, and analyzed before preparing the composite sample for cooperative work.

The Bordeaux, analyzed by the official electrolytic method, showed a copper content of 18.51%; the lead arsenate, analyzed by the official sulfate method, contained 63.67% of lead oxide; while the zinc arsenite, analyzed by the phosphate method, contained 57.13%, and by the mercury-thiocyanate method, 56.93% of zinc oxide.

The mixture submitted for cooperative work should contain, therefore, 4.63% of copper, 31.84% of lead oxide and 14.26% of zinc oxide. This differs from the

sample used in the 1920 work in that the lead oxide content is increased to twice the amount and the copper content is reduced to approximately one-half.

### LEAD OXIDE.

Weigh 1 gram of the powdered sample and transfer to a beaker. Add 5 cc. of hydrobromic acid (sp. gr. 1.31), and 15 cc. of hydrochloric acid (sp. gr. 1.19), and evaporate to dryness to remove arsenic; repeat the treatment; then add 20 cc. of hydrochloric acid (sp. gr. 1.19), and again evaporate to dryness. Heat the residue to boiling in 25 cc. of 2N hydrochloric acid, filter immediately to remove silica, and wash with hot water to a volume of 125 cc. Care must be taken to see that all lead chloride is in solution before filtering. If it will not all dissolve in 25 cc. of the 2N acid add 25 cc. additional and dilute the filtrate to 250 cc. volume. Pass in hydrogen sulfide until the precipitation is complete. Filter and wash the precipitate thoroughly with 0.5N hydrochloric acid saturated with hydrogen sulfide. Save the filtrate and washings for the determination of zinc. Transfer the filter paper containing the sulfides of lead and copper to a 400 cc. Pyrex beaker and completely oxidize all organic matter by heating with 4 cc. of concentrated sulfuric acid, together with a little fuming nitric acid; then completely remove nitric acid by heating on a hot plate to copious evolution of the white fumes of sulfuric acid, cool, add 2 or 3 cc. of water and again heat to fuming. Cool and determine the lead as sulfate as directed for lead arsenate[1], beginning with "Cool, add 50 cc. of water and about 100 cc. of 95% alcohol". The alcoholic solution should not stand more than 24 hours before filtering, as the solution may creep up the sides of the beaker and deposit crystals of copper sulfate which are very difficult to redissolve in the acid alcohol. From the weight of lead sulfate calculate the amount of lead oxide present, using the factor 0.73600.

### COPPER.

Evaporate the filtrate and washings from the lead sulfate precipitation to fuming, add a few cc. of fuming nitric acid to destroy organic matter, and continue the evaporation until about 3 cc. remain. Determine the copper by Low's titration method as directed under Bordeaux mixture[2], or by electrolysis as follows:

Take up the sulfuric acid solution with water, add 1 cc. of concentrated nitric acid, and filter if necessary. Make the volume to about 150 cc. and electrolyze as usual.

### ZINC OXIDE.

#### REAGENT.

Dissolve 27 grams of mercuric chloride and 39 grams of potassium thiocyanate in 1 liter of water. In lieu of the potassium thiocyanate, 30 grams of ammonium thiocyanate may be used[3].

#### DETERMINATION.

Concentrate the filtrate and washings from the sulfide precipitation by gentle boiling to about 50 cc.; continue the evaporation to dryness on a steam bath and dissolve the residue in 100 cc. of water containing 5 cc. of hydrochloric acid (1 to 1). Add 40 cc. of the mercury-thiocyanate reagent and stir vigorously until the zinc is precipitated. Allow to stand for at least an hour with occasional stirring, filter through a tared Gooch crucible, wash with water containing 20 cc. of the mercury-thiocyanate reagent per liter, and dry to constant weight at 105°C. From this weight calculate the zinc oxide, using the factor 0.16331.

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[1] *Assoc. Official Agr. Chemists, Methods*, 1920, 58.
[2] *Ibid.*, 62.
[3] *Trans. Am. Inst. Met.*, 1914, **8**: 146; *J. Am. Chem. Soc.*, 1918, **40**: 1036.

TABLE 3.

*Cooperative results on Bordeaux—lead arsenate—zinc arsenite.*

| ANALYST | LEAD OXIDE | COPPER | | ZINC OXIDE |
|---|---|---|---|---|
| | | Electrolytic Method | Titration Method | |
| | *per cent* | *per cent* | *per cent* | *per cent* |
| L. N. Markovitz | 31.58 | 4.65 | .... | 14.29 |
| | 31.85 | 4.61 | .... | 14.30 |
| | 31.28 | 4.62 | .... | 14.37 |
| | ..... | 4.64 | .... | ..... |
| Average | 31.57 | 4.63 | .... | 14.32 |
| H. L. Fulmer | 29.77 | .... | 4.75 | ..... |
| | 29.25 | .... | 4.75 | ..... |
| Average | 29.51* | .... | 4.75 | ..... |
| J. J. T. Graham | 31.80 | 4.64 | .... | 14.29 |
| | 31.90 | 4.70 | .... | 14.30 |
| | 31.82 | .... | .... | 14.27 |
| | 31.97 | .... | .... | ..... |
| | 31.71 | .... | .... | ..... |
| Average | 31.84 | 4.67 | .... | 14.28 |
| Percy O'Meara | 31.71 | .... | 4.46 | ..... |
| | 31.69 | .... | 4.59 | ..... |
| | 31.67 | ... | 4.66 | ..... |
| Average | 31.69 | .... | 4.57 | ..... |
| C. M. Smith | 31.92 | .... | 4.64 | 14.34 |
| | 31.96 | .... | 4.64 | 14.33 |
| | 31.81 | .... | 4.63 | 14.28 |
| | 31.96 | .... | 4.64 | 14.31 |
| Average | 31.91 | .... | 4.64 | 14.32 |
| F. L. Hart | 31.70 | .... | 4.51 | 14.28 |
| | 31.63 | .... | 4.56 | 14.36 |
| | 31.60 | .... | 4.54 | 14.30 |
| | 31.63 | .... | .... | 14.34 |
| Average | 31.64 | .... | 4.54 | 14.32 |
| General average | 31.75 | 4.64 | 4.61 | 14.31 |
| Calculated value | 31.84 | 4.63 | 4.63 | 14.26 |
| G. E. Miller† | 32.02 | .... | 4.64 | 14.23 |
| | 31.60 | .... | 4.67 | 14.21 |
| | 31.74 | .... | 4.57 | 14.30 |
| Average | 31.79 | .... | 4.63 | 14.25 |

*Not included in the general average.
†Received too late for tabulation.

NOTE.—Some iron is generally present and during the zinc determination it should be in the ferrous condition. In making the sulfide precipitation the hydrogen sulfide should be passed into the solution for a sufficient time to reduce the iron, in addition to precipitating the copper and lead. The zinc-mercury-thiocyanate precipitate normally is white, and it should not contain occluded ferric thiocyanate sufficient to give it more than a faint pink color.

## DISCUSSION.

The first three reports received on this sample showed the need of a slight modification in the method for zinc oxide. The results were from 0.5 to 3.0% below the value for zinc oxide calculated from the analysis of the zinc arsenite in the sample. Percy O'Meara reported difficulty in obtaining a precipitate. This difficulty was evidently caused by a solvent action of the ammonium chloride formed by the neutralization of the hydrochloric acid in the filtrate from the hydrogen sulfide precipitation. Experiments were then conducted with the view of modifying the method to eliminate this error. Substitution of sodium hydroxide for the ammonium hydroxide as the neutralizing agent produced somewhat better results. However, by carrying the evaporation to dryness all of the acid was expelled thereby avoiding the presence of salts due to its neutralization. With this modification results were obtained which agreed with the calculated value.

The method as modified was sent at once to all of the cooperators who had not reported, and all results for zinc oxide shown in Table 3 were obtained by following these modified directions. Examination of Table 3 shows that these results are very good, there being a maximum variation of only 0.1% among four analysts, while the general average of all their results varies only 0.05% from the calculated value. The results for copper are also good and check the calculated value for copper. The results for lead oxide with one exception agree and check with the calculated value. The one exception may have been due to the vagueness of the method as sent out, in regard to the amount of sulfuric acid to use in digesting the sulfides of copper and lead. This vagueness has been corrected, and the method as given in this report specifies the use of a definite quantity.

## LONDON PURPLE.

Very little work was done during 1921 on London purple. The 1920 sample was analyzed by one chemist whose results are given in the following table:

TABLE 4.

*Total arsenic calculated as arsenious oxide.*

| ANALYST | OFFICIAL IODINE METHOD* | DISTILLATION METHOD† | ZINC OXIDE SODIUM CARBONATE METHOD‡ |
|---|---|---|---|
| | *per cent* | *per cent* | *per cent* |
| M. Harris, Bureau of Chemistry, Washington, D. C. | 29.73<br>29.85<br>..... | 30.02<br>30.18<br>29.94 | 30.10<br>29.85<br>29.93 |
| Average | 29.79 | 30.05 | 29.96 |
| General average 1920 | 29.54 | 29.65 | 29.70 |

*Assoc. Official Agr. Chemists, Methods, 1920, 56.
†Ibid., 54.
‡J. Assoc. Official Agr. Chemists, 1921, 4: 397.

These results agree fairly well with those reported last year. The methods with the exception of the distillation method were also tested by the referee in 1919[1] with satisfactory results.

## PARIS GREEN.

No cooperative work was done during 1921 on Paris green. The results for arsenious oxide by the bromate method given in the 1919 and 1920 reports were very good. The referee recommends this method for final adoption by the association.

## MAGNESIUM ARSENATE.

The referee has considered methods for the determination of magnesium in magnesium arsenate. Since most commercial magnesium arsenate contains some calcium compounds the problem is presented of separating a small amount of calcium from a much larger quantity of magnesium. The ordinary method of separation by means of ammonium oxalate can not be used since, under these conditions, magnesium is precipitated along with the calcium. Hillebrand[2] recommends the modified Stolberg[3] method for the separation of small amounts of calcium from large quantities of magnesium. The referee has adapted this method to the determination of calcium and magnesium in magnesium arsenate. Very satisfactory results have been obtained by the following procedure:

Weigh 2.0 grams of the sample, transfer to a Pyrex beaker and evaporate twice with a mixture of 5 cc. of hydrobromic acid (sp. gr. 1.31), and 15 cc. of hydrochloric acid (sp. gr. 1.19). Add 25 cc. of sulfuric acid (1 to 4), and evaporate to copious evolution of sulfuric acid fumes. Oxidize any organic matter with a few drops of fuming nitric acid and continue the evaporation to dryness on a hot plate. Cool and add 3 cc. of water. Heat until the magnesium sulfate is all in solution, adding a few more drops of water if necessary. This should make a nearly saturated solution. Cool and add 100 cc. of methyl-ethyl alcohol mixture (90 cc. of methyl and 10 cc. of ethyl), stir, and let stand for 1 hour. Filter and wash with the methyl-ethyl alcohol mixture. All of the calcium sulfate will be in the residue and the magnesium sulfate in the filtrate.

Dissolve the residue in hot water and a little dilute hydrochloric acid, neutralize with ammonia and add sufficient acetic acid to make the solution contain about 2%; heat to boiling and precipitate the calcium with ammonium oxalate solution. Allow to stand 3 hours on a steam bath, filter and wash with hot water. Dissolve in 200 cc. of water containing 25 cc. (1 to 4) sulfuric acid, heat to 70°C. and titrate with standard permanganate solution. From this titration calculate the per cent of calcium oxide in the sample.

Evaporate the alcoholic filtrate to dryness, dissolve in water and a little hydrochloric acid and make to a volume of 200 cc. Transfer a 50 cc. aliquot to a beaker, add 5 cc. of hydrochloric acid and dilute to about 100 cc. Add 10 cc. of a 10% solution of ammonium phosphate and make just alkaline with ammonia; after standing 10 or

[1] *J. Assoc. Official Agr. Chemists*, 1921, 4: 397.
[2] U. S. Geol. Sur., Bull. 700, 143.
[3] *Zeit. angew. Chem.*, 1903, 17: 769.

15 minutes, add 15 or 20 cc. of strong ammonia and allow to stand at least 4 hours. Filter, wash with ammonia water (1 to 10), ignite and weigh as magnesium pyrophosphate.

In view of the fact that at present there is very little, if any, magnesium arsenate on the market, the association is hardly justified in continuing this work.

## SUGGESTIONS FOR FUTURE WORK.

It has been noticed by the referee and other chemists in the Insecticide and Fungicide Laboratory of the Bureau of Chemistry that occasionally the official distillation method for total arsenic gives erratic results, and in all cases these results are low when compared with those by the Gooch and Browning method.

Graham and Smith[1] have shown that the presence of nitrates or nitrites in the sample will cause the official distillation method to give low results, especially when some time is allowed to elapse between the distillation and the titration of the distillate. It is suggested that this effect may be due to the slow oxidation of arsenic in the distillate by nitrosyl chloride resulting from the interaction of the nitrates or nitrites with hydrochloric acid.

They have shown that a modification of the method of Jannasch and Seidel[2] will give accurate results in the presence of a considerable quantity of nitrates. In this method the procedure is the same as in the official distillation method, except that hydrazine sulfate and sodium bromide are used as the reducing agents, and a smaller quantity of hydrochloric acid is used in the distillation.

Since small amounts of nitrates are very frequently present in insecticides, it is important that the association take steps at once to so modify the present official distillation method as to provide for this condition. The following procedure is recommended by Graham and Smith:

### REAGENTS.

(a) *Starch indicator.*—Prepare as directed under Paris green[3].
(b) *Standard arsenious oxide solution.*—Prepare as directed under Paris green[3].
(c) *Standard iodine solution.*—Prepare as directed under Paris green[3].
(d) *Standard bromate solution.*—Prepare as directed under zinc arsenite, page 392.
(e) *Hydrazine sulfate and sodium bromide solution.*—Dissolve 20 grams of hydrazine sulfate and 20 grams of sodium bromide in 1 liter of dilute (1 to 4) hydrochloric acid.

### DETERMINATION.

Weigh an amount of the sample containing not more than 0.4 gram of metallic arsenic and transfer to a distilling flask. Add 50 cc. of the hydrazine sulfate and sodium bromide solution and close the flask with a stopper through which passes the stem of

[1] *J. Ind. Eng. Chem.*, 1922, 14: 207.
[2] *J. prakt. Chem.*, 1915, 91: 133.
[3] *Assoc. Official Agr. Chemists, Methods*, 1920, 53.

a dropping funnel. Connect to a well cooled condenser, the delivery end of which is attached to the system of flasks used in the official distillation method, omitting the third flask. Boil for 2 or 3 minutes and then add 100 cc. of concentrated hydrochloric acid by means of the dropping funnel and distil until the volume in the distilling flask is reduced to about 40 cc.; add an additional 50 cc. of concentrated hydrochloric acid and continue the distillation until the contents of the flask are again reduced to about 40 cc. Wash down the condenser, transfer the contents of the receiving flasks to a 1 liter graduated flask, make to volume and mix thoroughly. Pipet a 200 cc. aliquot to a 500 cc. Erlenmeyer flask, nearly neutralize with sodium hydroxide, finish the neutralization with sodium bicarbonate, add 4–5 grams in excess and titrate with standard iodine solution using starch solution as indicator; or to the 200 cc. aliquot add 10 cc. of concentrated hydrochloric acid and titrate with the standard bromate solution as described under zinc arsenite, page 392.

This method has been thoroughly tested in the Insecticide and Fungicide Laboratory of the Bureau of Chemistry on a large number of samples. In all cases the results were very satisfactory, and the method is now used for the analysis of all samples containing nitrates.

In view of the foregoing facts, the referee suggests the adoption of this method at once as a tentative method, and that it be studied further with a view to its adoption as an official method after it has been tested by cooperative work.

## RECOMMENDATIONS.

It is recommended—

(1) That the mercury-thiocyanate method for zinc oxide in zinc arsenite, page 392, be adopted as an official method. (Adopted as a tentative method in 1920.)

(2) That the bromate method, procedures (1) and (2), for the determination of arsenious oxide in zinc arsenite, page 394, be adopted as an official method.

(3) That the official method for the determination of water-soluble arsenic in lead arsenate[1] be adopted as official for the determination of water-soluble arsenic in zinc arsenite.

(4) That the bromate method, page 394, be adopted as an official method for the titration of the acid distillate in the official distillation method for the determination of total arsenic.

(5) That no further study be made of the modified Gooch and Browning method, page 395, for the determination of total arsenic in calcium arsenate.

(6) That the bromate method, procedures (1) and (2), for the determination of arsenious oxide in calcium arsenate, page 395, be adopted as an official method.

(7) That method (1), page 395, for the determination of calcium

[1] *Assoc. Official Agr. Chemists, Methods*, 1920, 59.

oxide in calcium arsenate be adopted as an official method. (Adopted as a tentative method in 1920.)

(8) That method (2), page 396, for the determination of calcium oxide in calcium arsenate be adopted as an official method. (Adopted as a tentative method in 1920.)

(9) That in the "General procedure for the analysis of a product containing arsenic, antimony, lead, copper, zinc, iron, calcium, magnesium, etc.," page 398, the methods for lead oxide and copper be adopted as official methods.

(10) That in the "General procedure for the analysis of a product containing arsenic, antimony, lead, copper, zinc, iron, calcium, magnesium, etc.," page 398, the method for zinc oxide be adopted as an official method. (Adopted as a tentative method in 1920.)

(11) That further action on the official distillation method for the determination of total arsenic in London purple be deferred until the suggested modification, page 402, has been studied.

(12) That the zinc oxide-sodium carbonate method be adopted as an official method for the determination of total arsenic in London purple.

(13) That the bromate method, procedures (1) and (2), for the determination of arsenious oxide in Paris green, as given in the referee's report in 1920, be adopted as an official method.

(14) That no further work be done at this time on magnesium arsenate.

(15) That the words "Not applicable in presence of nitrates" be placed over the present distillation method for total arsenic wherever it occurs among the methods of the association.

(16) That the distillation method for total arsenic in the presence of nitrates, page 402, suggested by Graham and Smith be adopted as a tentative method, with a view to its adoption as an official method after it has been further tested by cooperative work.

(17) That the work on insecticides and fungicides for 1922 be a study of the distillation method mentioned in Recommendation 16 for the determination of arsenic in the presence of nitrates.

# REPORT ON SOILS.

By W. H. MacIntire (Agricultural Experiment Station, Knoxville, Tenn.), *Referee.*

Your referee made an attempt to evolve a rapid and dependable method for the determination of the total sulfur in soils. Considerable preliminary work was done by the referee alone, after which a number of procedures were drafted and sent out to thirty of those from whom collaboration was expected. Though some of the procedures were considered inexact, they were incorporated in the outline in order that the referee's findings might be confirmed, since they included principles of technique which have been used in determining sulfates.

In the preliminary work, the original soil in each case was run in parallel with the same soil which had been fortified with either sodium, calcium or magnesium sulfate, in order to ascertain whether an accumulation of sulfates derived from the preliminary oxidative process could be recovered from the soil mass.

The preliminary work of the referee may be concisely given as follows:

(1) Charges of 10 grams each of five unfortified soils were incinerated with 10 grams of magnesium nitrate, and the mass was lixiviated and leached with distilled water. Concordant results could not be secured.

(2) A 25-gram charge of one soil was incinerated with 10 grams of ammonium nitrate. The residue was pestled and digested, and then leached through a Büchner funnel with water, hydrochloric acid (1 plus 9), nitric acid (1 plus 9) and acetic acid (1 plus 9). Both the water extract and the acetic acid extract yielded sulfate recoveries far in excess (about six times) of the recoveries given by the hydrochloric and nitric acid digestions and extractions of both native and fortified soils. The iron carried by the acid solutions was removed as insoluble oxides by evaporation to dryness and ignition upon an electric hot plate, after the addition of an excess of a base to hold the sulfate radicle. It afterwards appeared that the high heat necessary to convert all iron salts into insoluble oxides caused dissociation of the sulfate.

(3) Ten-gram charges of each of three soils were fused with a mixture of 15 grams of sodium carbonate and 5 grams of potassium carbonate, extracted with water and filtered. The residues were then extracted with hydrochloric acid in order to dissolve any barium carbonate. Dilute sulfuric acid added to these acid extracts yielded 0.006, 0.0038 and 0.0019 gram of barium sulfate, indicating that barium occurrences could not account for any appreciable part of the unrecovered added sulfate.

(4) Twenty-five-gram charges of each of four soils were incinerated with 25 grams of sodium peroxide. The residues were extracted with water or concentrated hydrochloric acid. The extracted residue was then dehydrated and again leached with water or hydrochloric acid. In no case did this procedure result in a recovery equivalent to the blank of the original soil plus the added sulfate. The recovery in each instance was considerably greater with the water extraction than with the hydrochloric acid extraction, in one instance about 25 times.

(5) Several soils were digested separately with a mixture of hydrochloric, nitric and hydrofluoric acid and with nitro-hydrofluoric acid. Great difficulty was experienced in removing all of the fluorine, resulting in contamination of the barium sulfate precipitates with barium fluoride.

(6) Twenty-gram charges of each of three soils were digested with 20 cc. of hydrofluoric acid and 25 cc. of nitric acid and filtered. The acid solutions were then evaporated and the siliceous residue ignited with magnesium nitrate. After ignition with magnesium nitrate the residues were subjected to separate extractions with water, hydrochloric acid and acetic acid, and the second extractions added to the respective initial acid extractions. The contaminative influence of barium fluoride previously noted was again experienced.

(7) Twenty-five-gram charges of each of three soils, both native and fortified, were ignited with magnesium nitrate and subjected to separate extractions with water, hydrochloric acid and acetic acid. In the case of one soil, the three solvents gave sulfate extractions which were about equivalent, while with the other two soils, the hydrochloric and acetic acid extractions were greater than those secured by water; all, however, were below the amount of sulfate added.

(8) The J. Lawrence Smith procedure, preceded by incineration with magnesium nitrate, was tried upon each of three soils, using 25-gram charges. After drying and heating the soil with a mixture of 5 grams of magnesium oxide and 11.5 cc. of nitric acid, a disintegration was effected by ignition of the residue with a mixture of 2 grams of ammonium chloride and 10 grams of calcium carbonate. The ignited residue was then boiled with distilled water, ammonium carbonate and bromine; filtered; concentrated to 400 cc., and barium sulfate determined. In this procedure, results secured upon the native soil were higher than those obtained by any of the other procedures; however, the recovery of added sulfates in the same three soils which had been fortified was in no case equivalent to the theoretical or expected recovery. It appears probable that the fault lay in the excessive heat utilized in the preliminary oxidation with magnesium nitrate in the electric furnace.

(9) Twenty grams of soil mixed with magnesium oxide and nitric acid, using a slight excess of magnesium oxide, were evaporated to dryness on an electric hot plate and then heated in an electric furnace. The incinerated mass was pestled and extracted with (1) water and (2) acetic acid. Five and four-tenths and 7.5 milligrams of barium sulfate were obtained, respectively. With added calcium sulfate equivalent to 0.1033 gram of barium sulfate, only 0.0388 gram and 0.0781 gram of barium sulfate were obtained by water and acetic acid, respectively, with digestions of thirty minutes at boiling temperature.

(10) Twenty-five-gram charges evaporated with a solution of 10 grams of ammonium nitrate on an electric hot plate, heated in an electric furnace, and the residue boiled thirty minutes with an excess of acetic acid, gave a recovery of 0.0895 gram of barium sulfate from soil plus sodium sulfate equivalent to 0.1033 gram of barium sulfate; while only 0.0836 gram of barium sulfate was recovered from the same soil, fortified with potassium sulfate of the same barium sulfate equivalence. A corresponding addition of $SO_4$, as magnesium sulfate, gave a recovery of but 0.0249 gram of barium sulfate for the fortified soil.

(11) Twenty-five grams of soil, fortified with 0.1033 gram of barium sulfate equivalent were boiled 1 hour with 150 cc. of nitric acid and filtered. The insoluble residue was then boiled with nitric acid (1 plus 9) containing 0.2500 gram of magnesium oxide and filtered. The combined filtrates were evaporated and heated, and the insoluble oxides boiled with acetic acid and removed by filtration. The total barium sulfate recovery from the fortified soil was only 0.0073 gram. The same treatment applied to a charge fortified with sodium sulfate equivalent to 0.1033 gram of barium sulfate gave only 0.0189 gram of barium sulfate.

As a result of the foregoing and additional preliminary work, six different procedures were sent to each of thirty collaborators, in order to ascertain whether the preliminary

findings in the referee's laboratory were to be found with other types of soil. Procedure VI was not carried out by any of the collaborators because of the inability to secure calcium peroxide.

REFEREE'S OUTLINE OF PROCEDURES FOR COLLABORATORS.

| | | |
|---|---|---|
| Charges | Sandy soils and other soils low in organic matter | 50 grams. |
| | Loams, peats and soils high in organic matter | 25 grams. |

Run one loam or clay loam and one soil of local interest by each procedure. In addition, fortify a charge of each soil with an aliquot of calcium sulfate or magnesium sulfate solution (preferably both) sufficient to give a precipitate of 0.2000 gram of barium sulfate, and compare the addition to the determined recovery from the fortified soil, minus that from the soil unfortified. Make all barium sulfate precipitations from a volume of about 200 cc. at boiling temperature, with slow addition of barium chloride and vigorous agitation. Permit the barium sulfate precipitate to stand in a warm place for 18 hours. Determine and apply the reagent blanks. Filter all barium sulfate precipitates upon an acid-washed asbestos filter in a platinum or Vitreosil crucible. Place the Gooch in a porcelain crucible and heat moderately for 10 minutes.

I. *Peroxide combustion.*—(a) Mix an air-dry charge of soil with 25 grams of sodium peroxide and then introduce sufficient water to insure a well-mixed thick paste. Transfer to a nickel or other suitable crucible and heat in the electric furnace for a full hour after expulsion of moisture at the extreme heat of the furnace. Remove incinerated mass by mechanical means; pulverize and transfer to a 500 cc. graduated flask (or liter flask, if need be). Add about 100 cc. of water and a few drops of bromine. Dissolve the alkali and add an excess of 25 cc. of hydrochloric acid (1 to 1). Boil for an hour, maintaining a volume of about 300 cc.; cool and make to volume. Pour off on a 9 cm. Büchner filter the supernatant liquid and the necessary fraction of the suspended matter to secure an aliquot of one-half the volume of the flask. Evaporate the filtrated aliquot to dryness. Take up with 10 cc. of hydrochloric acid (1 plus 9); remove silica along with iron by ammonia, electrolysis or conversion to insoluble oxide, as may be most feasible, and dissolve with acetic acid. (It is essential that the iron be removed.) Precipitate the barium sulfate in the manner prescribed and from the volume previously designated.

(b) Proceed as in (a) up to the point where the 1 to 1 solution of hydrochloric acid is added. Instead of acid, add distilled water and boil; filter; wash; acidulate the filtrate; remove the silica and determine the barium sulfate.

II. *Magnesium nitrate combustion.*—(a) Mix the charge with a saturated, slightly alkaline solution of magnesium nitrate to a thick workable paste. Dry and heat in the electric furnace for an hour after expulsion of water. Transfer the ignited residue to a 500 cc. Pyrex beaker. Add 300 cc. of distilled water; introduce a few drops of bromine and boil for 1 hour over sulfur-free heat. Throw the hot mixture on a 9 cm. Büchner filter and wash with hot water to filtrate volume of 1 liter. Concentrate to 400 cc. and precipitate the barium sulfate.

(b) Proceed as in (a) up to the point of boiling with distilled water. Instead of water, add an excess of acetic acid and boil for 1 hour. Filter; wash the precipitate with hot water to a filtrate volume of 1 liter. Concentrate filtrate to 400 cc. and precipitate barium sulfate.

(c) Proceed as in (a) up to the point of boiling with distilled water. Add an excess of hydrochloric acid and boil for 1 hour. Filter through a 9 cm. Büchner filter and wash the precipitate with hot water to a filtrate volume of 1 liter. Evaporate to dryness; dehydrate and remove the silica. Take up and remove iron. (See appended note.) Precipitate the barium sulfate.

III. *Aqua regia digestion.*—Introduce the charge into a 500 cc. Kjeldahl round-bottom flask. Add 50 cc. of concentrated nitric acid and then 100 cc. of concentrated hydrochloric acid. Introduce a small funnel into the neck of the flask. Clamp the flask in an upright position and boil gently for 1 hour. Cool; dilute to 400 cc. and filter through a double No. 2 Whatman filter on a Büchner funnel, leading into a 1-liter Pyrex beaker placed under a filtration bell jar. Wash the precipitate with hot water to a filtrate volume of 1 liter. If the soil is not rich in basic materials, add 0.5 gram of C. P. magnesium oxide to the acid filtrate and evaporate to dryness. Convert the ferric salts to oxides and remove both silica and iron as directed in the appended note. Evaporate to remove the excess of acetic acid used in taking up the ignited residue. Add 10 cc. of concentrated hydrochloric acid and again evaporate. Take up with 1 or 2 drops of hydrochloric acid and precipitate the barium sodium from a volume of 400 cc., as previously directed.

IV. *Nitric acid digestion.*—Introduce the charge into a 500 cc. Kjeldahl flask as in Procedure III. Add 150 cc. of concentrated nitric acid. Then boil and filter as in Procedure III. After the removal of the iron and the evaporation of the excess of acetic acid, take up with concentrated hydrochloric acid and evaporate once more in order to remove all nitrates before the precipitation of the barium sulfate.

V. *Magnesium nitrate-calcium carbonate-ammonium chloride combustion.*—To the soil charge add 5 grams of C. P. magnesium oxide and mix thoroughly in a platinum dish. Add 11.5 cc. of concentrated nitric acid in fractions, stirring with a small glass rod. Dry carefully with non-sulfur heat. Ignite in an electric furnace. Cool and mix the ignited residue with a pulverized mixture of 10 grams of pulverulent calcium carbonate and 2 grams of ammonium chloride. Heat in an electric furnace, continuing the heat for a period of 1 hour after the furnace has reached its maximum temperature. Cool; slake the lime and transfer the mass to a 600 cc. Pyrex beaker, pestling or policing and washing the fraction clinging to the side of the dish (using a few drops of acetic or hydrochloric acid, if need be) into the beaker. Add 400 cc. of distilled water and 15 grams of ammonium oxalate and a few drops of bromine. Heat on a sulfur-free heat to boiling for 1 hour. While hot, filter through a 9 cm. Büchner funnel and wash with hot water to a volume of 1.5 liters. Concentrate the filtrate to about 400 cc.; acidify with a slight excess of hydrochloric acid, and precipitate the barium sulfate as previously prescribed, being sure to agitate vigorously during the addition of barium chloride.

VI. *Calcium peroxide combustion.*—To the soil charge add and thoroughly mix 12.5 grams of powdered calcium peroxide (37.5 grams of $CaO_2 . 8 H_2O$). Heat slowly and then for an hour at full heat of an electric furnace. Cool; slake and continue as above under Procedure V, with reference to the treatment of the ignited residue.

Evaporate the acid solution in the 1-liter Pyrex beaker and heat till no trace of acid fumes can be detected. Allow the beaker to cool; add 10 cc. of acetic acid and 150 cc. of water; and boil for 15 minutes. Filter the granular, insoluble oxides on a small Büchner or Hirsh filter, washing with hot water. Add 10 cc. of concentrated hydrochloric acid to the filtrate, and evaporate off the acetic acid. Add 10 cc. of hydrochloric acid and repeat the evaporation; take up with a few drops of hydrochloric acid and hot water and filter off any silica present.

NOTE.—The iron in the nitric acid and hydrochloric-nitric acid solutions may be removed by insuring the presence of sufficient base to hold the sulfate radicle and then converting the ferric salts to insoluble oxides, thereby eliminating an excess of ammonium salts and the necessity of handling the ammoniacally precipitated hydrated oxide.

For convenience in consulting the tables the following index to the procedures is given:

Procedure I. *Peroxide combustion.*
(a) hydrochloric acid extraction.
(b) water extraction.

Procedure II. *Magnesium nitrate combustion.*
(a) water extraction.
(b) acetic acid extraction.
(c) hydrochloric acid extraction.

Procedure III. *Aqua regia digestion.*

Procedure IV. *Nitric acid digestion.*

Procedure V. *Magnesium nitrate-calcium carbonate-ammonium chloride combustion.*

Procedure VI. *Calcium peroxide combustion.*

TABLE 1.

*Comparison of methods for determination of sulfur.*

(Analyst, Charles Reeder)

PROCEDURE I.

| Soil | Barium Sulfate Added* | Total Barium Sulfate | Barium Sulfate in Soil | Barium Sulfate Recovered | Barium Sulfate Recovered | Iron Removed | Barium Sulfate in 12.5 Grams of Soil | Sulfur |
|---|---|---|---|---|---|---|---|---|
| *gs.* | *mgs.* | *mgs.* | *mgs.* | *mgs.* | *per cent* | | *mgs.* | *per cent* |
| 12.5† | | 16.0<br>16.1 | 16.1†† | | | No | 16.1 | 0.0178 |
| 10.5† | | 15.3<br>16.1 | 15.7†† | | | No | 18.7 | 0.0205 |
| 10.5† | 118.5 | 122.2<br>121.9 | 15.7†† | 106.3 | 89.7 | No | | |
| 12.5† | | 19.2<br>20.5 | 19.6†† | | | Yes | 19.6 | 0.0215 |
| 12.5† | | 20.2<br>19.1 | 19.5†† | | | Yes | 19.5 | 0.0214 |
| 6.5† | 84.7 | 92.1<br>90.8 | 9.7†† | 81.8 | 96.7 | Yes | | |

**Procedure II.**

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 25‡ | | 8.5<br>6.7 | 7.6 | | | | 3.8 | 0.0042 |
| 25§ | | 31.4<br>32.8 | 32.1 | | | | 16.1 | 0.0176 |
| 12 | | 21.6<br>21.1 | 21.3 | | | No | 22.1 | 0.0243 |
| 12 | 135 | 154.6<br>154.2 | 21.3 | 133.1 | 98.7 | No | | |
| 12 | | 2.4<br>2.8 | 2.6 | | | Yes | 2.7 | 0.0029 |
| 12 | 135 | 113.4<br>29.7 | 2.6 | | | Yes | | |
| 12 | | 1.0<br>2.0 | 1.5 | | | Yes | | |
| 12 | | 57.1<br>84.7 | 1.5 | | | Yes | | |

**Procedure III.**

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 12** | | 15.4<br>15.2 | 15.3†† | | | No | 16.0 | 0.0175 |
| 12** | 135 | 149.6<br>149.9 | 15.3†† | 134.5 | 99.8 | No | | |
| 12** | | 16.2<br>15.8 | 16.0†† | | | Yes | 16.7 | 0.0184 |
| 12** | 135 | 149.3<br>151.0 | 16.0†† | 134.2 | 99.7 | Yes | | |

**Procedure V.**

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 15‡ | | 12.7<br>12.4 | 12.5 | | | | 10.4 | 0.0115 |
| 15‡ | 169 | 128.3<br>129.0 | 12.5 | 116.2 | 68.8 | | | |
| 12† | | 4.4<br>10.7 | | | | Yes | | |
| 12† | 135 | 100.8<br>34.8 | | | | | | |
| 12† | | 20.3<br>21.1 | 20.7 | | | No<br>No | 21.5 | 0.0236 |
| 12† | | 154.4<br>155.9 | 20.7 | 134.5 | 99.9 | No<br>No | | |

*Equivalent from calcium sulfate added.
†Hydrochloric acid extraction.
‡Water extraction only.
§Acetic acid extraction.
**Aqua regia extraction.
††Average.

TABLE 2.

*Comparison of methods for determination of sulfur (expressed as sulfur trioxide).*

(Analyst, S. Lomanitz)

| Soil Number | Procedure I (Water Extraction) | Procedure II (Water Extraction) | Procedure IV | J. L. Smith Modified | Calcium Nitrate |
|---|---|---|---|---|---|
| | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* |
| 5954 | 0.128 | 0.001 | 0.060 | 0.082 | ..... |
| 1956 | 0.032 | 0.002 | 0.013 | 0.025 | 0.053 |
| 18910 | 0.064 | 0.006 | 0.020 | 0.050 | 0.035 |
| 18911 | 0.054 | 0.010 | 0.022 | 0.121 | 0.045 |
| 18999 | 0.055 | 0.029 | 0.011 | 0.004 | 0.067 |
| 19000 | 0.032 | 0.027 | 0.007 | 0.073 | 0.067 |

TABLE 2 (Continued).

*Calcium sulfate recovered from barium sulfate equivalent additions*.*

| Soil Number | Calcium Sulfate† | Calcium Sulfate‡ | Calcium Sulfate‡ | Calcium Sulfate† | |
|---|---|---|---|---|---|
| | *gram* | *gram* | *gram* | *gram* | |
| 5954 | 0.0610 | 0.0250 | 0.1200 | 0.0625 | |
| 1956 | 0.1350 | 0.1340 | 0.1830 | 0.0720 | |
| 18910 | 0.1350 | 0.1080 | 0.1740 | 0.1175 | |
| 18911 | 0.0825 | 0.1230 | 0.1360 | ..... | |
| 18999 | 0.0915 | 0.1235 | 0.1225 | 0.1110 | |
| 19000 | 0.1225 | 0.0850 | 0.1200 | 0.0625 | |

*After applying respective blanks from soil.
†Added 0.1340 gram of calcium sulfate.
‡Added 0.1883 gram of calcium sulfate.

TABLE 3.

*Comparison of methods for determination of sulfur (expressed as barium sulfate).*

(Analyst, A. L. Prince)

| Plat | Procedure I | Procedure II | Procedure III | Procedure IV |
|---|---|---|---|---|
| | *gram* | *gram* | *gram* | *gram* |
| 11 B | 0.0437* | ..... | 0.0120* | 0.0072* |
| 11 A | 0.0792† | 0.0396† | 0.0444† | 0.0501† |

*Corrected for blank and obtained from 10-gram sample.
†One determination.

TABLE 4.

*Comparison of methods for determination of sulfur (expressed as sulfur).*

(Analyst, V. H. Morris)

| Soil Number | Procedure I | | Procedure II | | | Procedure III | Procedure IV | Procedure V |
|---|---|---|---|---|---|---|---|---|
| | (a) | (b) | (a) | (b) | (c) | | | |
| | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* |
| 1* | 0.0127 | 0.0106 | 0.0105 | 0.0123 | 0.0115 | 0.0061 | 0.0089 | 0.0138 |
| 1† | 0.0382 | 0.0461 | 0.0605 | 0.0647 | 0.0641 | 0.0424 | 0.0400 | 0.0487 |
| 2‡ | 0.0441 | 0.0215 | 0.0195 | 0.0119 | 0.0135 | 0.0049 | 0.0411 | 0.0432 |
| 2† | 0.1263 | 0.0741 | 0.1229 | 0.1196 | 0.1222 | 0.0684 | 0.1400 | 0.1439 |

*Soil No. 1 is a Volusia silt loam low in organic matter.
†Magnesium sulfate equivalent to 0.1940 gram of barium sulfate was used to fortify these soils. The amount of sulfur which should have been obtained from Soil No. 1, fortified, is equal to 0.0659 per cent sulfur; Soil No. 2 is 0.1506 per cent sulfur.
‡Soil No. 2 is a sample of Clyde clay relatively high in organic matter.

TABLE 5.

*Comparison of methods for determination of sulfur (expressed as barium sulfate).*

(Analyst, W. M. Shaw)

| Soil and Treatment | Procedure III | Procedure III (Modified) | Procedure IV (Modified) | Procedure V |
|---|---|---|---|---|
| | *gram* | *gram* | *gram* | *gram* |
| Soil only* | 0.0049‡ | 0.0283 | 0.0325 | ..... |
| | 0.0095§ | 0.0303 | 0.0300 | ..... |
| | 0.0147 | ..... | 0.0285 | ..... |
| Average | 0.0097 | 0.0293 | 0.0303 | ..... |
| Soil plus magnesium sulfate† | 0.0405‡ | 0.1635 | 0.1649 | 0.0815** |
| | 0.0109§ | 0.1550 | 0.1530 | 0.1770†† |
| | 0.0387 | 0.1560 | ..... | ..... |
| Average | 0.0300 | 0.1582 | 0.1590 | ..... |
| Theoretical | 0.1387 | 0.1583 | 0.1593 | 0.1223 |

*Charge of 25 grams.
†Equivalent to 0.1290 gram of barium sulfate.
‡Iron removed by heating salts to convert into oxides.
§Iron removed by double precipitation with ammonium hydroxide.
**Minimum of 7 determinations.
††Maximum of 7 determinations.

TABLE 6.

*Recovery of precipitated barium sulfate from a concentrated nitric acid digestion*.*

| | | | |
|---|---|---|---|
| *Digestion of 0.1290 gram of barium sulfate only.* | | | |
| | *gram* | *gram* | *gram* |
| Residual from acid digestion | 0.0700 | 0.0515 | 0.0394 |
| Reprecipitated from filtrate | 0.0400 | 0.0485 | 0.0634 |
| Redissolved in dilute hydrochloric acid | 0.0105 | 0.0150 | 0.0171 |
| *Digestion of 0.1290 gram of barium sulfate plus 25 grams of soil.* | | | |
| Final recovery | 0.0450 | 0.0426 | 0.0425 |
| Same from soil only | 0.0325 | 0.0300 | 0.0285 |
| Recovery due to barium sulfate addition | 0.0125 | 0.0126 | 0.0140 |

*Iron eliminated by double ammoniacal precipitation.

## COMMENTS BY ANALYSTS.

*Charles Reeder, Oregon Agricultural Experiment Station.*—The modifications in the methods of procedure indicated in the following paragraphs were found to be necessary. We found time for work with but one soil, Willamette silt loam.

Although Procedure I (a) is cumbersome and time-consuming because of the large volume of washing from the iron precipitate, we are inclined to think it is by all odds the most reliable of the several proposed. For some reason, not yet understood, we could not get satisfactory results from Procedure II (c) (iron removed) or from Procedure V (with iron removed). Determinations by the same procedure with iron not removed are too high because of contamination with iron. Our filtrations were through asbestos fiber in porcelain, not Vitreosil crucibles. Details of the changes in the methods of procedure are indicated in the following paragraphs:

I: (a) 40 grams of sodium peroxide. Mix the sodium peroxide with moistened soil, as this prevents loss of soil with the escaping gases. Use centrifuge instead of Büchner; remove iron by ammonium hydroxide. (b) Changes as above except removal of iron.

II: (a) No change. (b) No change. (c) Instead of washing precipitate to volume of 1 liter make up to 1 liter; use centrifuge on aliquot. Remove iron with ammonium hydroxide.

III: Start the reaction of the acid on the soil slowly, using hot water, then a very low flame. Make to volume; take aliquot, using centrifuge. Remove iron by ammonium hydroxide.

V: Use a solution of magnesium nitrate.

*G. S. Fraps, Texas Agricultural Experiment Station.*—I regret that this report is not as satisfactory as we would desire for the reason that we were able to make only single determinations. While Mr. Lomanitz is an experienced analyst, some experience with the methods to be studied is also desirable. The recovery of an added sulfate was not satisfactory with any of the methods. To judge from the results of analysis, Procedure V was the most satisfactory of those tested. I have made regular determination of sulfur on these samples by the calcium nitrate method. Procedure II (a) was not satisfactory, as you can see by the figures. Procedure I (b), involving the use of sodium peroxide, is very long and tedious.

*A. L. Prince, New Jersey Agricultural Experiment Station.*—As the time which could be devoted to this work was limited, only a few determinations were made. The lack of an electric furnace precluded attempting some of the methods. However, the peroxide combustion and magnesium nitrate methods were tried out, using simply the full heat of the Bunsen burner.

In the determination 11 B, Table 3, the soil was derived from a plot treated with ammonium sulfate and lime, while 11 A was a soil from a plot treated with ammonium sulfate and no lime.

The peroxide method gave much higher results than aqua regia and nitric acid oxidations, even after subtracting the blank from the sulfur contained in the peroxide. In all of these methods, the iron was removed by means of ammonia. In the aqua regia and nitric acid methods, the digestion was carried out for 1 hour, the mixture cooled and filtered at once. In all cases, the barium sulfate precipitate was collected on an asbestos filter in a porcelain Gooch crucible.

The same methods were employed on soil 11 A and the magnesium nitrate method (c) was also tried out without use of the electric furnace. The results were higher on this soil, as would be expected. The peroxide determination, as in the case of 11 B, gave much higher results than the other methods. In the nitric acid oxidation, however, the material was not filtered for 2 days after the 1-hour digestion. This may

account for the slightly higher results in this case. With the exception of the peroxide method the checks in the other methods were very poor.

From the meagerness of this work and the poor checks obtained, it would be unwise to draw any conclusions. However, of the methods tried, there is some indication that the peroxide combustion, although quite lengthy, might prove more reliable for the determination of total sulfur in soils.

*V. H. Morris, Ohio Agricultural Experiment Station.*—Procedure I: (a) The extreme heat called for in this procedure is hardly deemed necessary for the oxidation of the sulfur. The term "extreme heat" was taken in this case to mean about 850°C. Such an ignition leaves a residue very difficult to remove from the crucible. Iron was eliminated in this procedure by precipitation as hydroxide in ammoniacal solution, the silica being dehydrated and removed previously. (b) Difficulty was encountered in this procedure in the enormous mass of silica which came out of solution on making the filtrate acid. This was true especially with the sample of soil low in organic matter, a charge of 50 grams being used. This method is not as effective as (a) in extracting the sulfur.

Procedure II: This method seems to be very effective particularly in removing the sulfur added as magnesium sulfate. However, it does not extract all the sulfur originally in the soil, this being shown in the soil sample high in organic matter.

Procedure III: Aqua regia does not appear effective. An attempt to remove the iron as oxide failed, perhaps due to insufficient heating. It was then removed as hydroxide, this method being used in all other cases where the elimination of iron was necessary. It is possible that there was some loss of sulfur at this point by occlusion, although two precipitations and washings of the hydroxide were made.

Procedure IV: Nitric acid appears to be much more effective with the soil sample high in organic matter. This may be due to the smaller charge.

Procedure V: Difficulty was encountered in this procedure owing, presumably, to the addition of ammonium oxalate. Upon concentrating the filtrate, heating with the oxalate, filtering and washing, a considerable quantity of a crystalline precipitate settled out. Qualitative tests proved this to be magnesium oxalate, although the oxalate of magnesium is soluble and should be, particularly in such a large volume and acid solution. An attempt was made to get the precipitate into solution; after decanting the clear solution, it was immediately reprecipitated. It was then necessary to remove the magnesium as magnesium ammonium phosphate. The solution required 15 cc. of hydrochloric acid to keep barium phosphate in solution when sulfates were precipitated. It is possible that a small amount of barium phosphate was included with the barium sulfate, since the results obtained in this procedure are slightly higher than those from the sodium peroxide fusion.

## DISCUSSION OF RESULTS.

Using the blank determination secured upon the native soil by the sodium peroxide procedure, results submitted by the Oregon Experiment Station show a recovery of but 89.7 per cent of the sulfur added to the soil by hydrochloric acid extraction of the residue when iron was not removed, and 96.7 per cent by the same procedure when iron was removed. The necessity for the removal of iron salts prior to the precipitation of barium sulfate is emphasized by these results and by those of C. B. Williams in 1902. With the magnesium nitrate combustion the recovery of sulfate from the untreated soil was greatest with the hydrochloric acid extraction, acetic acid and water following in the order named. Applying the hydrochloric acid blank to the recovery from the fortified soil the hydrochloric acid extraction without removal of iron gave a 98.7 per cent recovery from the addition of 0.1000 gram of calcium sulfate.

With the aqua regia digestion of two soils the checks were quite concordant, and when applied to the respective total recoveries the amount of sulfate yielded was 99.8 and 99.7 per cent, respectively, for the technique involving the removal of iron and that permitting its retention in the solution from which the barium sulfate precipitation was made. With the magnesium nitrate-calcium carbonate-ammonium chloride ignition the water and hydrochloric acid extractions gave checks in the respective procedures; but, when carried out with the fortified soil, the application of the blanks gave but 68.8 per cent of the added sulfate in the case of the water extraction as against 99.9 per cent for the hydrochloric acid extraction, the iron not having been removed from the latter.

Lomanitz found wide variations between the several methods as tried upon each native soil, the sodium peroxide fusion with water extraction and the magnesium nitrate-calcium carbonate-ammonium chloride procedures giving the highest results as compared with the calcium nitrate procedure in vogue at the Texas Experiment Station. However, when the respective checks upon the native soils were applied to the fortified soils, the highest average recovery was obtained by the nitric acid digestion. The nearest approach to any single recovery of added calcium sulfate was also obtained by the nitric acid digestion.

The results submitted by Prince showed a much higher determination by the sodium peroxide procedure than by the nitric acid and the aqua regia digestions for the fortified soil, when the iron was not removed by ammoniacal precipitation. This does not mean necessarily that oxidation was less complete in the two wet digestions than in the peroxide combustion method, but that the amount of sulfur leached from the soil mass was less. However, from other work, it would seem probable that the lower results were caused by the loss of sulfate during the reduction of the ferric salts to insoluble oxides.

The data submitted by the Ohio Experiment Station may be used to stress some of the findings of the referee as to the variations in the tendencies of the several procedures when applied to different soils. In the case of the sodium peroxide fusion with Soil No. 1, Table 4, a greater amount of barium sulfate precipitate was obtained by the hydrochloric extraction of the fusion of the native soil than by the water extraction. However, the reverse was true for this soil after it had been fortified with additions of magnesium sulfate. The same experience was had by the referee in working with this procedure.

With the magnesium nitrate procedure the greatest recovery was obtained from the acetic acid extraction for both native and fortified soil low in organic matter. On the other hand, in the case of the soil high in organic matter, the highest recovery from the unfortified soil was obtained by the water extraction while the three extractions—water, hydrochloric acid and acetic acid—gave practically equivalent results when this soil was fortified by sulfate additions. The aqua regia digestion gave low results when heating was resorted to in an effort to remove iron (followed in this case by ammoniacal precipitation). This result, also obtained by the referee, was shown to be due to the dissociation of the sulfates, particularly magnesium sulfate, carried by the strong acid filtrate. The nitric acid digestion gave interesting and promising results, especially in the case of the soil high in organic matter, the recovery being greater than that secured by the use of sodium peroxide and practically equivalent to that secured by following the magnesium nitrate-calcium carbonate-ammonium chloride procedure. The magnesium nitrate-calcium carbonate-ammonium chloride procedure gave results as a rule higher than those secured by any of the other methods. The average of the four determinations by this method was 0.0624 gram of barium sulfate as compared to 0.0575 gram of barium sulfate for a corresponding average by the nitric acid digestion and 0.0553 gram of barium sulfate for the sodium peroxide ignition with hydrochloric acid.

Morris states that the results secured by the magnesium nitrate-calcium carbonate-ammonium chloride procedure may be too high because, "it is possible that a small amount of barium phosphate was included with barium sulfate". As offsetting this error, it should be noted that the strong solution of hydrochloric acid, from which the barium sulfate was precipitated, would have had some appreciable solvent action upon the barium sulfate.

Morris experienced trouble with a continued precipitation of magnesium oxalate. W. M. Shaw of the referee's laboratory encountered the same trouble. In the preliminary work with this procedure, the referee used ammonium carbonate instead of ammonium oxalate to effect removal of calcium and magnesium, and no trouble was experienced in the use of the carbonate. It was thought best to substitute the oxalate for the carbonate because the former could be more accurately added, since a considerable amount of the carbonate undergoes decomposition upon adding it to the warm solution.

Since a number of analyses carried out in the preliminary studies by the referee had demonstrated that the sodium peroxide procedure would not effect recovery of even an approximation of added sodium sulfate and magnesium sulfate in the case of local soils, Shaw did not carry out this procedure. His results indicate complete recovery of added sulfates in the case of both the aqua regia and the nitric acid digestions.

Closely concordant, separate and average sulfate results were obtained by the aqua regia and the nitric acid digestions where iron was removed by ammoniacal precipitation, but it was apparent that the excessive heat required to convert the ferric salts to iron oxides was responsible for either a volatilization or dissociation of the sulfate or else a markedly decreased solubility of the sulfates in the acid solvents.

In the case of the fortified soil the results were low and non-concordant for both the magnesium nitrate-calcium carbonate-ammonium chloride procedure and the aqua regia digestion when iron was removed by heating; but both nitric acid and aqua regia digestions gave theoretical recoveries when the iron was removed by ammoniacal precipitations.

## POSSIBLE LOSS OF SULFATES IN THE NITRIC ACID PROCEDURE, DUE TO BARIUM NATIVE TO THE SOIL.

It is a well-known fact that some soils carry an appreciable amount of barium compounds which might vitiate the sulfate result obtained by methods calling for acid digestion. It was found that some of the soils studied at the Tennessee Experiment Station contained this element. Since the nitric acid digestion procedure appears well adapted because of its simplicity and rapidity, it seemed worth while to test the possible influence of the occurrence of native barium upon the accuracy of the method. The data secured demonstrate that all of the added sulfates can be recovered, when applying the soil blank to the determination of the fortified soil, if iron is removed by double ammoniacal precipitation; hence, the undetermined factors are the possible interference of barium and the question of the completeness of the oxidation of all forms of native organic sulfur within the period of boiling. It is planned to continue the studies as to completeness of oxidation in the given time, using 1-hour, 2-hour and 3-hour digestions.

In an effort to obtain light upon the tendency of the siliceous residues to occlude sulfur, as barium sulfate, Shaw introduced freshly-precipitated barium sulfate into the soil mass, prior to the 1-hour period of digestion with nitric acid, and compared the sulfate recoveries with those from the unfortified soil. He also ran the barium sulfate precipitate without soil as a further parallel. The steps of digestion, evaporation of the nitric acid extract, the elimination of nitrates by repeated evaporations with hydrochloric acid, the precipitation of iron ammoniacally, the taking up with an amount of

hydrochloric acid necessary to bring all salts into solution and the neutralization of this excess of hydrochloric acid prior to the addition of barium chloride, were all carried out as in a normal procedure with an unknown. The results show an average carry-through of 0.0142 gram of barium sulfate for the 0.1290 gram blank and an average of 0.0131 gram for the soil to which barium sulfate was added. Such a recovery would indicate, rather strongly, that the method is not appreciably affected by amounts of barium which are probably greater than those to be expected as native to the soil, the amount of the barium sulfate impregnation in this case being equivalent to an occurrence of 0.03% barium, on the basis of a 25-gram charge. The peroxide ignition method gave varying results with different soils and inconsistent recoveries with water and the several acid extractions. The amounts of salts present at the time of the precipitation of barium sulfate were excessive and dehydration of the siliceous mass was required to obviate occlusion. Some of the data obtained also indicate the probability of error as a result of uncontrolled temperature during the ignition.

In the case of the magnesium nitrate procedure it is apparent that variations in the temperature of the electric furnace are reflected in the results. It is quite possible, as indicated by numerous data obtained, that the magnesium sulfate may undergo dissociation during the ignition process.

The magnesium nitrate-calcium carbonate-ammonium chloride procedure has given satisfactory results in some instances, but it does not appear to be applicable to all soils. It is doubtful if this or either of the other dry methods effects an absolute oxidation of the soil sulfur, for each has in some instances yielded hydrogen sulfide when the alkaline ignition residue was treated with hydrochloric acid.

The nitric acid digestion is preferable to the aqua regia digestion because of the smaller amount of dissolved iron to be removed and the absence of the objectionable fumes incident to the aqua regia procedure. It is evident that complete recovery may be expected unless inhibitive amounts of barium should come into solution from the soil during the digestion. It is furthermore established by many data that the iron may not be eliminated through conversion of the iron salts into soluble oxides by excessive heat, without bringing about a dissociation of the sulfates and loss of sulfur trioxide, even when an abundance of the alkali or alkali-earth bases is added.

The intention was to secure a method of procedure which would accomplish two things: (1) A complete conversion of all forms of sulfur to the sulfate radicle; (2) the complete removal of all sulfates from the soil mass. It is essential, furthermore, to have a procedure which is adapted to a larger bulk of the original soil because of the low sulfur content in many soils. It is also desirable, if not in fact essential, that the insoluble residue shall be in such condition as to facilitate filtration and washing, and that the extract be not of excessive salt concentration. The wet method seems decidedly preferable in caring for this essential factor.

## RECOMMENDATION.

It is recommended that the following method be studied further:

Introduce 50 grams of soil low in organic matter, or 25 grams of soil high in organic matter, into a 500 cc. Kjeldahl flask, heat slowly and boil for 1 hour. Insert a small funnel in the neck of the flask. Follow the same procedure by boiling for a 2-hour period and also for 3 hours. Cool, dilute to 400 cc. and pour off the clear liquid through a Büchner funnel. Add 250–300 cc. of hot water, agitate, throw upon Büchner and wash with hot water to a combined volume of 1 liter. Evaporate filtrate to dryness at low temperature. Add 10 cc. of concentrated hydrochloric acid and again evaporate. Repeat the addition of and evaporation with hydrochloric acid. Take up with a few drops of hydrochloric acid; bring into solution and precipitate iron, by addition of 1 to 1

ammonium hydroxide, from a volume of 400 cc. Pour onto a Büchner and wash twice. Transfer the filter to original beaker, dissolve, macerate the filter and again precipitate from a volume of about 300 cc. and filter into original filtrate, washing to a volume of 1 liter. Acidify filtrate with a slight excess of hydrochloric acid; concentrate to a volume of 400 cc.; add hot barium chloride (1 plus 9) and agitate vigorously. Permit barium sulfate to stand 18 hours and filter on an acid-washed asbestos Gooch filter.

NOTE.—In studying this method it would be well to add a *small amount of freshly precipitated barium sulfate* to the soil prior to the digestion and determine the point at which it may be lost to the procedure, in order to ascertain what may be expected from any barium sulfate formed during the digestion because of the occurrence of barium compounds native to the soil. The method should also be tested by the addition of known amounts of sodium, potassium, calcium and magnesium sulfates.

## REPORT ON SULFUR.

By W. H. MACINTIRE (Agricultural Experiment Station, Knoxville, Tenn.), *Associate Referee.*

The referee was instructed to study sulfur as it relates to soil problems. The writer and W. M. Shaw of the same laboratory directed their work toward the thesis of the chemically induced formation of sulfates from flowers of sulfur as compared to that induced by biological agencies. This laboratory work was done with quartz media and it was run in parallel with lysimeter studies. Preliminary papers have been published[1]. A more extensive report will be offered in the near future.

## REPORT ON FOODS AND FEEDING STUFFS.

By J. B. REED (Bureau of Chemistry, Washington, D. C.), *Referee.*

The referee considered the different methods of determining the presence of sulfur dioxide in bleached grains and found that the one generally used is that by W. P. Carroll[2]. As this method has not been found entirely satisfactory, D. A. Coleman and his associates of the Bureau of Markets, Department of Agriculture, have studied various other methods. It is their opinion that the method of treating the sample with a non-volatile acid, such as phosphoric or tartaric, distilling and collecting the distillate in an acidified solution of potassium iodate to which starch has been added, is much better than the Carroll method. However, it has not been tried out by a sufficient number of chemists to warrant its recommendation for adoption as an official method.

[1] *Soil Science*, 1917, 4: 231; 1921, 11: 249; *J. Ind. Eng. Chem.*, 1921, 13: 310.
[2] U. S. Bur. Plant Ind. Circ. 40: 1909.

Methods for the determination of acidity in corn have been considered, and a comparative study of the Black and Alsberg[1] and the electrolytic hydrogen ion concentration methods has been made by C. D. Garby[2]. The work of L. H. Bailey and C. Thom[3] and others indicates that the method of Black and Alsberg is satisfactory for all practical purposes for determining acidity in grains, and it requires less complicated apparatus and less skill in manipulation than the electrolytic hydrogen ion concentration method. However, as little is known of the nature of the changes which take place in corn and other grains as the acidity increases and owing to the fact that it has been found by Bailey and others that the acidity is not as definitely indicative as has been thought, it does not seem desirable to recommend any method as official until the changes which take place are better understood, and the degree of acidity has greater significance.

The existing official method for determining water in foods and feeding stuffs has been considered, and the conclusion is that several official methods should be adopted, the method to be used depending upon the nature of the product under examination. In each case the simplest and most rapid accurate method for any given product should be used. In the opinion of the referee the method of drying a weighed sample to constant weight in a suitable dish on the water bath should be used wherever possible on account of its simplicity. Next in simplicity is the method of drying in a vacuum at the boiling point of water. It should be used with products the moisture of which can not be determined satisfactorily by the first method.

The moisture in some products can not be determined at the boiling point of water on account of certain changes which take place at that temperature which affect the results. With products of this kind the determination should be made by heating in vacuum at from 65 to 70C°. It has not been decided just what temperature will give the most satisfactory results. Some products, however, can not be heated to 65° in vacuum without vitiating the results of the moisture determination. The moisture of products of this kind should be determined by drying in a desiccator in vacuum over sulfuric acid.

The problem which presents itself is to decide what method for the determination of moisture shall be used on the various foods and feeding stuffs, that is, to classify the products as to the method which should be used in the determination of moisture in them. It will be necessary to get the opinion of many food analysts. Though this whole problem may be difficult, it seems possible that a fairly satisfactory classification may be worked out in time.

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[1] U. S. Bur. Plant Ind. Bull. 199: (1910), 10.
[2] Thesis, "Electrometric Titration and its application to Corn Meal", 1921. May be consulted at George Washington University Library.
[3] The Operative Miller, 1920, 25: 368.

The methods for the detection of reground bran and shorts presented in 1920 by J. B. Reed[1] and D. B. Bisbee[2] have been studied further and although they may be valuable as methods for detecting ground bran in shorts, it is believed that neither one of them taken alone is sufficiently reliable in the hands of those who have not had considerable experience with it so that the referee can recommend its adoption as official.

The motion made by G. S. Fraps that the words "or asbestos" be inserted after the word "linen" in the official crude fiber method has been considered, and it is believed that this point should be disposed of in connection with the report on crude fiber.

The motion by G. S. Fraps that the first and second sentences in the official method for the determination of ether extract[3] be deleted has been considered by the referee. In view of the facts that it was necessary to adopt the Roese-Gottlieb[4] method for the determination of ether extract in dairy products and that a modification of that method is being used in the Bureau of Chemistry on noodles, macaroni and like products, it is possible that the present official method for the determination of ether extract may not be entirely satisfactory for all the foods and feeding stuffs on which it is being used. Therefore it would seem desirable to make a more extended study of the subject to determine whether it will be necessary to make fundamental changes in the method. If that is found necessary, it will be easier to make all of the changes at one time.

## RECOMMENDATIONS.

It is recommended—

(1) That the method of treating the samples with a non-volatile acid, such as phosphoric or tartaric, distilling and collecting the distillate in an acidified solution of potassium iodate, to which starch has been added, be studied further in comparison with the Carroll and other methods.

(2) That no method for determining the acidity in corn and other cereals be adopted at present.

(3) That the referee attempt to classify products according to the method which should be used in determining moisture in them; that the various methods be studied and simplified by rewording; and that the conditions of temperature, pressure and other factors be fixed more definitely.

(4) That the referee study methods of determining ether extract in various foods and feeding stuffs the coming year, with a view to ascertaining whether or not the official method is applicable to all of the products for which it is now being used.

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[1] *J. Assoc. Official Agr. Chemists*, 1921, 5: 70.
[2] *Ibid.*, 74.
[3] *Assoc. Official Agr. Chemists, Methods*, 1920, 227.
[4] *Z. Nahr-Genussm.*, 1905, 9: 531.

# REPORT ON CRUDE FIBER.

By G. L. BIDWELL (Bureau of Chemistry, Washington, D. C.), *Referee.*

At the meeting of this association in 1920 a paper, entitled "A Study of the Details of the Crude Fiber Method", was presented by G. L. Bidwell and L. E. Bopst. This paper contained a proposed method for crude fiber which gives concordant results if followed exactly. During 1921 additional study of this method was made. A copy was sent to practically all of the collaborators for criticism and reports. As a result of careful examination some valuable suggestions were offered. Many of these suggestions were incorporated in a rewritten method which, it is hoped, will meet with the approval of the association. The method follows:

## REAGENTS.

(a) *1.25% sulfuric acid solution.*—Contains 1.25 grams of sulfuric acid per 100 cc.

(b) *1.25% sodium hydroxide solution.*—Contains 1.25 grams of sodium hydroxide per 100 cc., free, or nearly so, from sodium carbonate.

The strength of these solutions must be accurately checked by titration.

*Asbestos.*—First digest on steam bath over night with 5% to 10% sodium hydroxide and thoroughly wash with hot water; then digest over night with 5% to 10% hydrochloric acid and again wash thoroughly with hot water; next ignite completely at bright red heat.

## APPARATUS.

*Water-jacketed condenser* (about 15 inch).

*Assay flask.*—Capacity about 700 cc., diameter of base 3½ inches, 7½ inches tall, and tapering to fit a No. 10 rubber stopper. In case the assay flask is not available a 500–750 cc. Erlenmeyer flask may be used.

*Linen.*—Linen should be of such character that while filtration is rapid no solid matter passes through. The linen which has proved most satisfactory has 46x50 threads per inch. The threads are of large size as compared to the number of threads per inch and are loosely twisted. Any linen approximating these specifications will prove satisfactory.

## DETERMINATION.

Extract 2 grams of the dry material with ordinary ether, or use the residue from the ether extract determination and transfer the residue, together with ½ to 1 gram of asbestos, to the assay or Erlenmeyer flask. (Where the residue from the ether extract is used and the proper amount of asbestos has already been added, further addition is unnecessary.) Using a calibrated beaker, add 200 cc. of boiling sulfuric acid (a) to the contents of the flask, place immediately on the heating battery and connect with the water-cooled condenser. It is essential that the contents of the flask come to boiling within 1 minute after being placed upon the battery and that the boiling continue briskly for 30 minutes. It was found best to rotate the flask with the hand about every 5 minutes in order thoroughly to mix the charge. Care should be taken to keep the sides of the flask above the solution free from the sample. A blast of air conducted into the flask will serve to reduce the frothing of the liquid. Remove flask at the expiration of the 30 minutes and immediately filter through linen in a fluted funnel and wash with boiling water until the washings are no longer acid.

Next wash the charge and adhering asbestos back into the flask with 200 cc. of boiling sodium hydroxide solution (b) using a 200 cc. wash bottle. (The transfer of the sample to the original container is very easily accomplished by this means.) Bring the sodium hydroxide to boiling and keep at this temperature under a reflux condenser while in use. (The sodium hydroxide is best transferred to a 200 cc. wash bottle by means of a bent tube through which the liquid is forced by blowing into a tube connected with the top of the condenser.) Then place the flask in the heating battery, connect with the reflux condenser and boil for exactly 30 minutes. The boiling with the alkali should be so timed that the contents of the different flasks will reach the boiling point approximately 3 minutes apart. This provides sufficient time for filtration. The last filtration takes place directly into a Gooch crucible, which has been prepared previously with a thin but close layer of ignited asbestos. Employ suction and wash the contents thoroughly with hot water and then with about 15 cc. of 95% alcohol.

Dry the crucibles with their contents to constant weight at 110°C. in an electric oven, usually overnight. After weighing, incinerate the contents of the crucibles in an electric muffle or on a Meker burner at a dull red heat until the carbonaceous matter has been removed—20 minutes is usually sufficient. Cool in a small, tight, efficient desiccator and weigh. The loss in weight is taken as crude fiber.

It is recommended that this method be adopted by the association.

A lengthy discussion followed this report in view of the change suggested in the official method. The referee reported that the addition of asbestos was practically the only difference and emphasized the point that the variation in results was not due so much to the method itself as to the details followed by the collaborators in running the method.

## A STUDY OF THE GEPHART METHOD FOR THE DETERMINATION OF CRUDE FIBER.

By Leslie E. Bopst and George L. Bidwell (Bureau of Chemistry, Washington, D. C.).

A method for the determination of crude fiber, primarily intended for that determination in cocoa, was developed by F. C. Gephart, a consulting chemist of New York. This method was studied with the cooperation of Gephart to see if it had any possibilities as a general crude fiber method. The characteristics which made it attractive were that it needed no condenser, did not foam and required no filtering. The method is as follows:

Weigh out directly into the special silica tube from ½ to 1.0 gram of the material. Add 40 cc. of ether, stir thoroughly and centrifuge for 5 minutes at a speed of 3000 revolutions per minute. Carefully pour off the supernatant ether and repeat the operation with a second portion of 40 cc. of ether. Dry the tube and contents, add 40 cc. of boiling 1.25% sulfuric acid and digest in a boiling water bath for 30 minutes, stirring the contents of the tube frequently with a glass rod provided with a hooked end. Centrifuge for 10 minutes at the same speed, pour off supernatant liquid, add 40 cc. of boiling water, stir and centrifuge for 10 minutes, repeating the washing operation with a second portion of hot water. Add 40 cc. of boiling 1.25% sodium hydroxide and digest in

boiling water for 30 minutes. Centrifuge as before, wash with 2 portions of boiling water, and finally with 40 cc. of a 50–50 mixture of alcohol and ether. Dry at 105°C. to constant weight, ignite and weigh. The difference in weight is taken as crude fiber.

As this method was first used in cocoa and chocolate work by Gephart, it was thought advisable to try it upon the same type of samples.

Fiber was determined upon cocoas of different standards of purity by the Gephart method and by the proposed method with the following results:

TABLE 1.

*Determinations of crude fiber in cocoa.*

| QUALITY OF COCOA | PROPOSED METHOD | GEPHART'S METHOD |
|---|---|---|
| | *per cent* | *per cent* |
| Standard | 5.83 | 5.51 |
| High grade | 4.85 | 4.97 |
| Standard | 5.35 | 5.91 |

The figures in Table 1 show that the Gephart method gives slightly higher results (with one exception) than the proposed method. This is probably due to the fact that filtration after acid digestion removes some of the material which can not be eliminated by Gephart's method.

These methods were also tried upon samples of widely varying fiber content with the following results:

TABLE 2.

*Determinations of crude fiber in various feeding mixtures.*

| SAMPLES | PROPOSED METHOD | GEPHART'S METHOD |
|---|---|---|
| | *per cent* | *per cent* |
| Alfalfa, corn meal and cottonseed meal | 13.90 | 14.58 |
| Alfalfa-corn meal | 8.62 | 8.87 |
| Grain dust (low fiber content) | 9.03 | 9.66 |
| Grain dust (high fiber content) | 23.20 | 25.55 |
| Corn cob cellulose | 61.45 | 66.68 |
| Wheat flour | 0.28 | 0.40 |
| Cottonseed meal | 8.25 | 10.95 |

It is apparent from these results that the greater the amount of fiber in a sample the greater the range of difference between the two methods. Samples of low-fiber content with the exception of flour check fairly well, but the variance between the results increases as the fiber content increases.

It is thought that this method will be of value in the case of samples having the texture of cocoa and spices which are very difficult to filter when no asbestos is used. However, if the proposed method for the determination of crude fiber be adopted, very little difficulty will be experienced in filtering any material.

## REPORT ON STOCK FEED ADULTERATION.

By H. E. GENSLER (Bureau of Chemistry, Department of Agriculture, Harrisburg, Pa.), *Associate Referee.*

As associate referee on stock feed adulteration, the writer has developed a method for the quantitative determination of grit and bone in poultry feeds and animal by-products.

The method follows:

*Estimation of grit in poultry feeds and other similar feeds.*

After thoroughly mixing, place 2 grams of the ground sample prepared for the determination of protein, fat and fiber, or 2 grams of a representative portion of the original sample, ground to pass through a mm.- or a 20-mesh sieve, in an evaporating dish of about 30 cc. capacity.

Add about 5 cc. of chloroform and mix gently with a glass rod so that the liquid comes in contact with all of the sample. Brush the particles adhering to the rod into the dish and, after pushing all particles into the chloroform with a 25-mm. circular or square cover-glass, use the latter to skim off and pull the floating portion of the material over the top of the dish, taking care not to submerge the cover-glass enough to disturb the grit settled in the bottom of the dish. After skimming until the surface of the chloroform is rather clear, slowly pour the supernatant liquid into a second evaporating dish, stopping the pouring as soon as any grit threatens to pass out.

Now wash the sides of the dish with a few cc. more of chloroform and repeat the above operation until no floating particles remain, using the cover-glass as before and pouring off the supernatant liquid. This will require from 10–15 cc. of chloroform. When grit only remains, drain out chloroform and allow to dry. Weigh to constant weight.

WEIGHT OF GRIT $\times$ 50 = PER CENT OF GRIT.

NOTE.—The chloroform washings collected in the second dish should be poured out in order to observe whether any grit has been poured into it during the process. If any number of tests are to be made, the chloroform washings may be saved and recovered by distillation and subsequent drying over calcium chloride.

*Estimation of bone in meat scrap.*

The method for the estimation of grit in poultry feeds is employed, except that in some samples it may be found necessary to rub the residue of bone, remaining after washing with chloroform, with a glass rod or small pestle to assist in bringing some of the particles to the surface of the chloroform.

WEIGHT OF BONE $\times$ 50 = PER CENT OF BONE.

Eight samples were prepared to be sent to the collaborators, four of which were mixtures of unknown ingredients, submitted for the purpose

of qualitative identification of these ingredients as well as the determination of grit in the same by means of the suggested method.

The remaining four were samples of animal by-products, containing various proportions of bone, submitted for the purpose of applying the proposed method in estimating the percentage of bone present.

The poultry feeds, in the case of Samples 1, 2, 3 and 4, were compounded from a mixture of corn, oats, wheat, barley, buckwheat, kafir and sunflower seed, containing also a trace of rye and flaxseed, the mixture being ground to pass a 20-mesh sieve. In addition to the ground grains, the 4 samples also contained ground grit and locust bean meal, as follows:

(1) 1% of grit and 10% of locust bean meal (latter declared).
(2) 3.5% of grit and 0.5% of locust bean meal.
(3) 5% of grit and 5% of locust bean meal.
(4) 8.2% of grit and no locust bean meal.

The locust bean meal was used to give practice in the utilization of a standard in microscopic examinations, as well as to show ability to detect small amounts of ingredients.

Samples 5, 6, 7 and 8 were meat and bone meal products ground to pass a 20-mesh sieve. Sample 5 was a factory product, containing 15.7 per cent of bone, and was used as a base in preparing Sample 6, which contained 35.8 per cent of bone. Samples 7 and 8 were prepared from a commercial meat product containing, originally, no bone, but later diluted so as to contain 8.4 per cent and 21.0 per cent of bone, respectively. As indicated, bone was added to these samples in varying amounts and in presenting the figures due consideration was given to the fact that the bone included 4.35 per cent of moisture, 2.68 per cent of residue and non-extractive matter, and 8.90 per cent of ether extract. Therefore, the results obtained, as well as those reported by the collaborators, were considered to represent pure bone.

These 8 samples, together with a copy of the proposed method and instructions for examination, were sent to 21 collaborators who had signified their desire to cooperate in the work. Results were received from 12 collaborators and tabulated.

The results reported by the collaborators in the determination of grit in ground feeds were remarkably close to the theoretical amounts in each of the samples. The widest variation, as will be noted, amounted to only 1.0 per cent in one case—Sample 4. The average results were also very close to the theoretical amounts in the mixture. O. B. Winter, of Michigan, reported the following as having been obtained by the use of carbon tetrachloride: Sample 1, 1.1 per cent; Sample 2, 3.1 per cent; Sample 3, 5.5 per cent; Sample 4, 8.4 per cent; Sample 7, 8.8 per cent and Sample 8, 21.8 per cent, in addition to the results obtained by the use of chloroform.

TABLE 1.

*Determination of grit in ground feeds.*

| ANALYST'S NO. | SAMPLE 1, 1.0%* | SAMPLE 2, 3.5%* | SAMPLE 3, 5.0%* | SAMPLE 4, 8.2%* |
|---|---|---|---|---|
| | *per cent* | *per cent* | *per cent* | *per cent* |
| 2 | 1.4 | 3.9 | 5.1 | 8.3 |
| 3 | 1.1 | 3.7 | 5.0 | 8.0 |
| 6 | 1.1 | 3.5 | 4.8 | 8.3 |
| 7 | 1.6 | 3.7 | 5.4 | 8.0 |
| 8 | 1.4 | 3.9 | 5.7 | 8.5 |
| 10 | 1.1 | 3.5 | 4.9 | 7.7 |
| 12 | 0.9 | 3.4 | 4.5 | 7.4 |
| 14 | ... | ... | ... | ... |
| 16 | 1.0 | 3.3 | 4.4 | 7.2 |
| 17 | 1.1 | 3.8 | 4.6 | 8.6 |
| 19 | 1.0 | 3.4 | 4.5 | 7.9 |
| 21 | 1.1 | 3.3 | 4.7 | 7.6 |
| Average..... | 1.6 | 3.6 | 4.9 | 8.0 |

*Actual percentage of grit present.

TABLE 2.

*Determination of bone in meat products.*

| ANALYST'S NO. | SAMPLE 5, 15.7%* | SAMPLE 6, 33.8%* | SAMPLE 7, 8.4%* | SAMPLE 8, 21.0%* |
|---|---|---|---|---|
| | *per cent* | *per cent* | *per cent* | *per cent* |
| 2 | ... | ... | ... | ... |
| 3 | 14.8 | 35.5 | 8.3 | 20.3 |
| 6 | 14.2 | 33.3 | 7.9 | 19.4 |
| 7 | 14.0 | 33.7 | 8.2 | 20.5 |
| 8 | 16.2 | 35.7 | 8.6 | 21.6 |
| 10 | 16.1 | 37.1 | 8.6 | 22.0 |
| 12 | 13.7 | 32.9 | 8.3 | 20.7 |
| 14 | 14.8 | 35.7 | 8.8 | 22.0 |
| 16 | 15.1 | 34.2 | 8.2 | 19.4 |
| 17 | 14.9 | 36.3 | 8.8 | 19.9 |
| 19 | 15.6 | 36.1 | 8.6 | 21.5 |
| 21 | 15.1 | 36.5 | 8.8 | 20.8 |
| Average..... | 14.9 | 35.2 | 8.5 | 20.8 |

*Actual percentage of bone present.

In the determination of bone in meat products, the results obtained were also in very close agreement with the theoretical amounts. The widest variations reported were 2.0 per cent in Sample 5; 2.9 per cent in Sample 6; 0.5 per cent in Sample 7 and 1.6 per cent in Sample 8. The latter figures, as previously explained, were obtained by the use of the factor which took into account constitutents other than actual bone, applied to the percentage of bone employed in making the mixtures.

These results would indicate the practicability of using such a method in estimating the amounts of grit or bone in certain feeds.

The results reported covering the identification of ingredients in the 4 samples of mixed feed were very good, indicating that one trained in

TABLE 3.

*Identification and relative amount of ingredients compared to standard*.*

| ANALYST'S NO. | CORN | OATS | WHEAT | BARLEY | RYE (Trace) | BUCK-WHEAT | MILO OR KAFIR | FLAX-SEED (Trace) | LOCUST BEAN, 10% (Standard for Sample 2, 0.5%; Sample 3, 5%; Sample 4, None) |
|---|---|---|---|---|---|---|---|---|---|
| Sample 1 | | | | | | | | | |
| 2 | S T | S T | S T | S T | S T | S T | S T | .... | Found present. |
| 3 | S T | T | T | S T | .... | T | .... | T | Found present. |
| 8 | S T | S T | S T | S T | S | S T | S T | .... | Found present. |
| 12 | S T | s | S T | S T | .... | S T | S T | .... | Found present. |
| 17 | T | .... | .... | .... | T | .... | .... | .... | Found present. |
| 19 | S T | S T | S T | S T | .... | S T | S T | .... | 10%. |
| Sample 2 | | | | | | | | | |
| 2 | S T | S T | S T | S T | .... | S T | S T | .... | Less than Sample 3. (Estimated 1%). |
| 3 | S T | T | T | S T | T | T | .... | T | Less than Sample 1. |
| 8 | S T | S T | S T | S T | S t | S T | S T | .... | 0.5%. |
| 12 | S T | s | S T | S T | .... | S T | S T | .... | Small amount—less than 5%. |
| 17 | T | .... | T | .... | .... | .... | .... | .... | About 1%. |
| 19 | S T | S T | S T | S T | .... | S T | S T | t | Less than 10%. |
| Sample 3 | | | | | | | | | |
| 2 | S T | .... | S T | S T | .... | S T | S T | .... | Less than Sample 1. (Estimated 5%). |
| 3 | S T | S T | T | S T | T | .... | .... | T | More than Sample 1. |
| 8 | S T | S T | S T | S T | t | S T | S T | .... | 5%. |
| 12 | S T | s | S T | S T | .... | S T | S T | .... | About same as Sample 1. |
| 17 | .... | .... | .... | .... | .... | T | .... | .... | About 5%. |
| 19 | S T | S T | S T | S T | .... | S T | S T | .... | More than Sample 1. |
| Sample 4 | | | | | | | | | |
| 2 | S T | S T | .... | S T | S T | S T | S T | .... | Less than Sample 2. (Estimated0.25%). |
| 3 | S T | T | T | S T | .... | S | .... | .... | Less than Sample 1. |
| 8 | S T | S T | S T | S T | S | S T | S T | .... | Bare trace. |
| 12 | S T | s | S T | S T | .... | S T | S T | .... | None. |
| 17 | .... | T | .... | .... | .... | .... | .... | .... | None. |
| 19 | S T | S T | S T | S T | .... | S T | S T | t | None. |

S—Starch detected. T—Tissue detected.
s—Trace of starch detected. t—Trace of tissue detected.
*Analyst 19 also found a trace of sunflower seed in Sample 4.

this line of work can easily identify the ingredients of a feed. Practically every ingredient was identified by the collaborators.

The reports on the detection and estimation of locust bean meal are especially interesting. One analyst reported the exact amounts in Samples 2 and 3, while another made an estimate close to the figures for the same samples. Other analysts made comparisons, which indicate that while it is difficult to determine the exact amount of one ingredient, such as locust bean meal, it is often possible to form an opinion as to the approximate amount of an ingredient. When it is remembered that Sample 2 contained locust bean meal in such small quantity that it would amount to only ten pounds per ton, the ability of the collaborators to detect it indicates that small amounts of ingredients can usually be identified. However, it is considered by analysts as well as control officials that when an ingredient is claimed it should be present in amounts readily identifiable.

It would appear desirable at this time to recommend that some method, such as above presented, be studied further with the object of deciding its worthiness for official adoption. The close results obtained by Winter with the use of carbon tetrachloride suggests the advisability of further study with liquids having a specific gravity similar to the one employed by your referee this year.

While it may not be practicable to attempt to develop the work to such an extent that analysts can give final statements in the determination of amounts of ingredients in feed mixtures, the work of this, as well as of previous years, points to the belief that approximations can be made. It would be considered advisable to encourage the collaborators of the several States to take part in the work so that the reports on identifications, as well as on quantitive determinations of ingredients in feeding stuffs, may be made more uniform.

It is recommended—

That further study be made of the methods described in the associate referee's report for the estimation of grit in poultry feeds and other similar feeds and for the estimation of bone in meat scrap.

The appointment of the following committees was announced by the president:

*Committee on nominations:* R. W. Balcom of Washington, D. C., R. N. Brackett of South Carolina and H. C. Lythgoe of Massachusetts.

*Committee on resolutions:* Wm. Frear of Pennsylvania, Julius Hortvet of Minnesota and Miss B. H. Silberberg of Washington, D. C.

*Committee on auditing:* J. W. Kellogg of Pennsylvania and J. J. T. Graham of Washington, D. C.

*Committee to wait upon Secretary of Agriculture:* R. E. Doolittle of Illinois, B. B. Ross of Alabama and E. M. Bailey of Connecticut.

*Committee to wait upon the Honorary President:* B. B. Ross of Alabama, F. P. Veitch of Washington, D. C., and H. D. Haskins of Massachusetts.

## FIRST DAY.

# MONDAY—AFTERNOON SESSION.

## REPORT ON SACCHARINE PRODUCTS[1].

By H. S. PAINE (Bureau of Chemistry, Washington, D. C.), *Referee.*

Reports have been received from all the associate referees. J. F. Brewster was appointed to succeed F. W. Zerban, who was unable to continue as Associate Referee on Sugar House Products. C. H. Jones was appointed Associate Referee on Maple Products. S. F. Sherwood and O. S. Keener have continued their work on honey and maltose products, respectively.

It is gratifying to note from this year's reports that considerable progress has been made. In most cases, however, the collaborative work has not been carried far enough to warrant recommending that definite action on the methods be taken at this time. Accordingly, it is hoped that the investigations of saccharine products, now well under way, may be actively continued.

## DETECTION OF ARTIFICIAL INVERT SUGAR IN HONEY[1].

By SIDNEY F. SHERWOOD (Bureau of Plant Industry, Washington, D. C.), *Associate Referee.*

The resorcin and the aniline chloride tests for the detection of commercial invert sugar sirup in honey were adopted as tentative methods[2] on the recommendation of F. L. Shannon[3] who, as Associate Referee on Honey, had investigated numerous tests. His investigations did not extend to honeys that had been heated to comparatively high temperatures and, as question has been raised regarding the value of the tests in the case of heated honeys, it was considered advisable to extend the investigation to include honeys which had been so treated.

In the processes ordinarily used for the manufacture of commercial invert sugar, inversion is accomplished by heating sucrose solutions to which a very small percentage of acid has been added. If solutions of d-fructose are heated to a high temperature, especially in the presence of

[1] Presented by C. F. Walton, Jr.
[2] *Assoc. Official Agr. Chemists, Methods*, 1920, 112.
[3] *J. Assoc. Official Agr. Chemists*, 1916, 2: 169.

acid, the sugar is decomposed with the formation of more or less oxymethyl-furfural. Therefore commercial invert sugar sirup which has been manufactured by processes requiring heating ordinarily affords a positive reaction with these tests. The amount of furfural present may be large or small, depending upon conditions of manufacture, and no conclusions can be drawn from the tests as to the amount of the adulterant present.

C. A. Browne[1] states: "With natural honeys which have been boiled or heated to a high temperature for any great length of time, traces of furfural are formed just as in the invert sugar sirups, and these honeys will then react with the aniline reagent. The boiling of honeys, however, is a most questionable operation at best, the flavor of the honey being completely destroyed by this process of cooking. Careful producers and bottlers of honey take extreme pains in putting up their products not to expose the extracted honey to a temperature exceeding 80°C. (176°F.). A positive reaction of a honey with aniline acetate may, therefore, be regarded as an almost certain indication of adulteration with invert sugar". E. F. Phillips[2] states that honey should never be liquefied by direct application of heat, and that it should never be heated to a temperature greater than 160°F. (71.1°C.), as honey that is heated to 180°F. (82.2°C.) loses flavor and becomes darker in color.

The value of the resorcin, aniline chloride and other tests has been widely investigated, especially in Germany, and the following comments, with regard to the resorcin test, are typical of the conclusions. H. Kretzschmar[3] examined a large number of commercial honeys and indicates the reliability of the test. M. H. Quantin[4] concludes that in honey expressed or handled in ordinary manner at ordinary temperatures the presence of furfural indicates the presence of a foreign material, and that the presence of furfural in honey prepared by heating is not sufficient proof of the presence of commercial invert sugar. O. Lüning[5] concludes that the value of the resorcin test is much lessened in the case of heated honeys. In the case of two samples, heated for two hours at 80–85°C., he obtained a slight, quickly disappearing, red color. Heating the two samples to 80–85°C. for two hours and then keeping them at 60–50°C. for eighteen hours, he obtained in one case a strong red color and in the other a weak one. He refers to the investigations of Fiehe and Stegmüller[6] who heated honeys for four hours at 70°, 80°, 85°, and 90°C. and obtained only a faint, quickly disappearing, red color. (A positive test is indicated by an immediate orange-red color changing at once to a cherry or dark red. This color persists for hours.)

---

[1] U. S. Bur. Chem. Bull. **110**: (1908), 68.
[2] U. S. Bur. Entom. Bull. **75**: 1907, Part I.
[3] *Z. Nahr. Genussm.*, 1914, **28**: 84.
[4] *Ann. Chim. anal.*, 1910, **15**: 299.
[5] *Z. Nahr. Genussm.*, 1915, **29**: 117.
[6] *Arb. Kais. Gesundh*, 1912, **40**: 336.

The samples of honey used in the present investigation were as follows:

| SAMPLE NO. | NAME | ACIDITY |
|---|---|---|
| | | *per cent* |
| 1 | Tupelo | 0.12 |
| 2 | Clover | 0.19 |
| 3 | Clover* | 0.41 |
| 4 | Honeydew (Hawaiian) | 0.20 |

*Contained added tartaric acid: 2 grams to 500 grams of honey.

The acidity was determined by titration with 0.1N sodium hydroxide[1], and is expressed as "per cent free acid as formic". The acidity, on this basis, of 100 samples of American and Hawaiian honeys (including honeydew honey) is, maximum 0.25, minimum 0.04[1]; that of 72 samples of honey from Cuba, Mexico, and Haiti is, maximum 0.43, minimum 0[2].

Series A.—Heated for 1 hour at 160°F. (71.7°C.).

Series B.—Heated for ½ hour at 180°F. (82.2°C.).

Series C.—Heated for 20 minutes at 208–209°F. (97.8 to 98.3°C.).

Series X.—Original Tupelo honey plus 20% of commercial invert sugar sirup prepared according to the method of Herzfeld[3].

The directions followed in making the tests were identical with those given in Methods of Analysis[4]. A positive reaction in the case of the resorcin test is an *orange-red* color appearing *immediately* and quickly turning to cherry or dark red. In the case of the aniline chloride test it is a *bright red* color appearing *at once*.

The collaborators were J. M. Webre, Temtor Corn and Fruit Products Co., St. Louis, Mo.; C. G. Church, Fruit and Vegetable Chemistry Laboratory, Bureau of Chemistry, Los Angeles, Calif.; C. P. Wilson, Citrus Exchange By-Products Laboratory, Corona, Calif.; and A. V. Fuller, Service Division, American Sugar Refining Co., 117 Wall St., New York City.

The reports of the collaborators are as follows:

[1] U. S. Bur. Chem. Bull. 110: (1908), 49.
[2] *Ibid.*, 154: (1912), 12.
[3] U. S. Bur. Chem. Bull. 110: (1908), 64.
[4] *Assoc. Official Agr. Chemists, Methods*, 1920, 112.

TABLE 1.

*Resorcin Test.*

(Bryan's modification of Fiehe's test.)

SERIES A.

(Heated for 1 hour at 160°F.—71.7°C.)

| Sample No. | Time | Webre | Church | Wilson | Fuller | Sherwood |
|---|---|---|---|---|---|---|
| 1 | Immediate | Slight trace | Negative | Negative | Negative | Negative |
| | 1 minute | Strong trace | Negative | Negative | Negative | Negative |
| | 5 minutes | Strong trace | Negative | Negative | Negative | Negative |
| | Immediate | Doubtful | Negative | Negative | Negative | Negative |
| 2 | 1 minute | Doubtful | Negative | Negative | Negative | Negative |
| | 5 minutes | Doubtful | Negative | Negative | Negative | Negative |
| | Immediate | Doubtful | Negative | Negative | Negative | Negative |
| 3 | 1 minute | Slight trace | Negative | Negative | Negative | Negative |
| | 5 minutes | Pink | Negative | Negative | Faint pink | Negative |
| | Immediate | Doubtful | Negative | Negative | Negative | Negative |
| 4 | 1 minute | Doubtful | Negative | Negative | Faint yellow | Negative |
| | 5 minutes | Doubtful | Negative | Negative | Faint yellow | Negative |

SERIES B.

(Heated for ½ hour at 180°F.—82.2°C.)

| | | | | | | |
|---|---|---|---|---|---|---|
| | Immediate | Negative | Negative | Negative | Negative | Negative |
| 1 | 1 minute | Slight trace | Negative | Negative | Negative | Negative |
| | 5 minutes | Positive | Negative | Negative | Faint yellow | Negative |
| | Immediate | Negative | Negative | Negative | Negative | Negative |
| 2 | 1 minute | Negative | Negative | Negative | Pink | Negative |
| | 5 minutes | Doubtful | Negative | Negative | Faint pink | Negative |
| | Immediate | Slight trace | Negative | Negative | Negative | Negative |
| 3 | 1 minute | Trace | Negative | Negative | Pink | Negative |
| | 5 minutes | Strong trace | Negative | Negative | Pink | Negative |
| | Immediate | Doubtful | Negative | Negative | Negative | Negative |
| 4 | 1 minute | Slight trace | Negative | Negative | Negative | Negative |
| | 5 minutes | Pink-positive | Negative | Negative | Very faint pink. | Negative |

SERIES C.

(Heated for 20 minutes at 208 to 209°F.—97.8 to 98.3°C.)

| | | | | | | |
|---|---|---|---|---|---|---|
| 1 | Immediate | Negative | Negative | Negative | Negative | Negative |
| | 1 minute | Trace | Negative | Negative | Negative | Negative |
| | 5 minutes | Positive | Negative | Negative | Very faint pink. | Negative |
| 2 | Immediate | Negative | Negative | Negative | Negative | Negative |
| | 1 minute | Trace | Negative | Negative | Pink | Negative |
| | 5 minutes | Positive | Negative | Negative | Pink | Negative |
| 3 | Immediate | Slight trace | Negative | Negative | Negative | Negative |
| | 1 minute | Trace | Negative | Negative | Decided pink | Negative |
| | 5 minutes | Positive | Negative | Negative | Decided pink | Negative |
| 4 | Immediate | Slight trace | Negative | Negative | Negative | Negative |
| | 1 minute | Trace | Negative | Negative | Negative | Negative |
| | 5 minutes | Positive | Negative | Negative | Decided yellow | Negative |

SERIES X.

(Original Tupelo honey plus 20% of commercial invert sugar sirup prepared according to method of Herzfeld*.)

| | | | | | | |
|---|---|---|---|---|---|---|
| X | Immediate | Orange red | Negative | Negative | Negative | Cherry red |
| | 1 minute | Cherry red | Pink | Negative | Negative | Cherry red |
| | 5 minutes | Dark red | Pink | Negative | Negative | Dark red |

*U. S. Bur. Chem. Bull 110: (1908), 64.

TABLE 2.

*Aniline chloride test.*

(Feder's)

SERIES A.

(Heated for 1 hour at 160°F.—71.7°C.)

| Sample No. | TIME | WEBRE | CHURCH | WILSON | FULLER | SHERWOOD |
|---|---|---|---|---|---|---|
| 1 | Immediate | Negative | Negative | Negative | Darkening | Negative |
| | 1 minute | Negative | Negative | Negative | Darkening | Negative |
| | 5 minutes | Trace | Negative | Negative | Darkening | Negative |
| 2 | Immediate | Negative | Negative | Negative | Pinkish | Negative |
| | 1 minute | Negative | Negative | Negative | Brownish | Negative |
| | 5 minutes | Negative | Negative | Negative | Brownish | Negative |
| 3 | Immediate | Slight trace | Negative | Negative | Pinkish | Negative |
| | 1 minute | Slight trace | Negative | Negative | Pinkish | Negative |
| | 5 minutes | Slight trace | Negative | Negative | Reddish brown | Negative |
| 4 | Immediate | Positive | Negative | Negative | Pinkish | Negative |
| | 1 minute | Positive | Negative | Negative | Pinkish | Negative |
| | 5 minutes | Positive | Negative | Negative | Brown | Negative |

SERIES B.

(Heated for ½ hour at 180°F.—82.2°C.)

| | | | | | | |
|---|---|---|---|---|---|---|
| | Immediate | Slight trace | Negative | Negative | Slight pinkish | Negative |
| 1 | 1 minute | Slight trace | Negative | Negative | Pinkish | Negative |
| | 5 minutes | Slight trace | Negative | Negative | Brown | Negative |
| | Immediate | Negative | Negative | Negative | Pink | Negative |
| 2 | 1 minute | Negative | Negative | Negative | Pink | Negative |
| | 5 minutes | Negative | Negative | Negative | Brown | Negative |
| | Immediate | Slight trace | Negative | Negative | Decided pink | Negative |
| 3 | 1 minute | Slight trace | Negative | Negative | Distinct pink | Negative |
| | 5 minutes | Trace | Very slight pink | Negative | Brown | Negative |
| | Immediate | Negative | Negative | Negative | Pinkish | Negative |
| 4 | 1 minute | Negative | Negative | Negative | Brownish | Negative |
| | 5 minutes | Negative | Negative | Negative | Brown | Negative |

SERIES C.

(Heated for 20 minutes at 208 to 209°F.—97.8 to 98.3°C.)

| | | | | | | |
|---|---|---|---|---|---|---|
| | Immediate | Negative | Negative | Negative | Brownish | Negative |
| 1 | 1 minute | Negative | Negative | Negative | Brown | Negative |
| | 5 minutes | Negative | Negative | Negative | Brown | Negative |
| | Immediate | Negative | Negative | Negative | Pinkish | Negative |
| 2 | 1 minute | Negative | Negative | Negative | Pinkish | Negative |
| | 5 minutes | Negative | Negative | Negative | Brown | Negative |
| | Immediate | Slight trace | Negative | Negative | Decided pink | Negative |
| 3 | 1 minute | Positive | Very slight pink | Negative | Pink | Negative |
| | 5 minutes | Positive | Very slight pink | Negative | Brown | Negative |
| | Immediate | Slight trace | Negative | Negative | Pinkish brown | Negative |
| 4 | 1 minute | Positive | Negative | Negative | Brown | Negative |
| | 5 minutes | Positive | Negative | Negative | Brown | Negative |

SERIES X.

(Original Tupelo honey plus 20% of commercial invert sugar sirup prepared according to method of Herzfeld*.)

| | | | | | | |
|---|---|---|---|---|---|---|
| | Immediate | Positive | Negative | Negative | No report | Positive |
| X | 1 minute | Positive | Very slight pink | Negative | No report | Positive |
| | 5 minutes | Positive | Very slight pink | Negative | No report | Positive |

*U. S. Bur. Chem. Bull. 110: (1908), 64.

## Examination of the results shows:

*Resorcin test.*—Four of the collaborators obtained negative results in every case. One of them (Webre) obtained doubtful results in several cases and slight traces in several cases. In view of the fact that he reports one as "pink-positive" it would appear

that his "slight traces" refer to pink color. As noted previously, this does not constitute a positive test. It is of note that, in the sample to which invert sugar was added, Church, Wilson and Fuller obtained negative results.

*Aniline chloride test.*—Three of the collaborators obtained negative results in every case. One of them (Webre) obtained positive results in Sample 4, Series A (heated for 1 hour at 160°F.) and in Sample 4, Series C, (heated for 20 minutes at 208–209°F.) but obtained negative results in Sample 4, Series B (heated for ½ hour at 180°F.). He also reports positive results in Sample 3, Series C (heated for 20 minutes at 208–209°F.), after standing one minute. Fuller obtained traces of pink in several cases, but this does not constitute a positive test. Webre stated that the reaction taking place in five minutes in the case of dark-colored samples does not develop sufficient color to permit of accurate conclusions. Church stated that the very slight pink colors noted could be detected only in thin layers, and that the test is scarcely sharp enough to justify condemning a honey on the strength of this determination alone. It is of note that in the sample to which invert sugar was added Church and Wilson obtained negative results.

## CONCLUSIONS.

In the case of honeys heated at temperatures which would prevail in the ordinary commercial handling of this product, neither the resorcin nor the aniline chloride test affords results that can be construed to indicate the presence of commercial invert sugar sirup. Thus, a positive result with either test serves to indicate the presence of a foreign substance. It is considered that an insufficient number of reports have been obtained—though efforts were made to secure the assistance of a larger number of collaborators—to justify the above conclusions being regarded as final, and it is recommended that the work be repeated. If this is done, the directions sent to the collaborators should include a more complete and detailed description of the technique and of the color tints than is given in the official methods[1]. In spite of detailed description, it is obvious that the operator must have had experience in order to obtain correct results.

Referring to the possibility of obtaining a positive test in the case of a honey that has been grossly overheated, the associate referee wishes to present the question of whether the term "honey" may be applied properly to a product resulting from grossly overheating honey with resulting loss of flavor, darkening of color and production of furfural.

[1] *Assoc. Official Agr. Chemists, Methods*, 1920, 112.

## REPORT ON MAPLE PRODUCTS[1].

By C. H. JONES (Agricultural Experiment Station, Burlington, Vt.), *Referee.*

Your referee has had no opportunity during the past year to do any cooperative work on the Canadian lead number and the conductivity value of maple products.

Both procedures have been printed[2].

Your referee recommends that these methods be not adopted as tentative at this time.

## REPORT ON MALTOSE PRODUCTS.

By O. S. KEENER (Bureau of Chemistry, Washington, D. C.), *Associate Referee.*

The work on maltose in recent years has been in connection with the determination of maltose and dextrine in beer. However, since maltose products may contain both dextrose and maltose, in addition to dextrine, it seems very desirable that a method for the determination of both these sugars in the presence of dextrine be adopted.

A study of the literature for available methods indicated the following possibilities:

I. The use of maltase.

II. The use of maltase-free yeasts.

III. The use of ordinary yeasts (present provisional method).

IV. The determination of dextrine by precipitation with alcohol.

The use of maltase is prohibited, for the present, by the lack of an available supply of this enzyme. The difficulty of maintaining maltase-free strains of yeast, together with the care and time required in their use, also would seem to eliminate this method from ordinary use for the present. The use of ordinary yeast is unsatisfactory from a number of standpoints. Consequently it was decided to investigate further the determination of dextrine by precipitation with alcohol, with the subsequent determination of sugars in the filtrate by the usual combination of polariscopic and copper reduction methods.

To this end, considerable experimental work was done to ascertain the best conditions for the precipitation of the dextrine. This work has been practically completed, and the method is about ready for distribution to the collaborators.

It is recommended that this work be continued for another year.

---

[1] Presented by O. S. Keener.

[2] *J. Assoc. Official Agr. Chemists.*, 1921, 4: 428.

# REPORT ON SUGAR-HOUSE PRODUCTS[1].

By J. F. BREWSTER (Louisiana Sugar Experiment Station, New Orleans, La.), *Associate Referee.*

Pursuant to the recommendations[2] made by the Associate Referee on Sugar-House Products for 1919, F. W. Zerban, the cooperative work on ash determination was continued.

Samples of cane sirup, first molasses and final molasses were sent to a number of chemists who had signified their willingness to cooperate, but, up to the time of filing this report, an insufficient number of results had been returned to enable the associate referee to draw conclusions.

In submitting the samples to the various chemists it was recommended that in making the direct ash determinations four temperatures be tried—475°, 500°, 525° and 550°C. These temperatures range from the dullest red heat at about 475° to a very decided red at 550°. Both methods I and II of Zerban's report[3] were recommended to be tried.

Only one complete set of results is at hand. These were submitted by W. G. Raines, Jr., of the Louisiana Sugar Experiment Station. Many collaborators were compelled to forego cooperation on this phase of the work because their laboratories did not possess the necessary pyrometers or furnaces for careful measurement and control of temperature.

Three sets of results on sulfate ash were received, but since the object of making these determinations is to work out a factor for deduction by comparison with direct ash, the purpose will not be well served until many more results by both methods are at hand.

It is hoped that sufficient data from which definite conclusions may be drawn will be received from other collaborators before the next report is in order.

It should be pointed out that in shipping samples of sirup or molasses deterioration is fairly certain to follow. The proper time for cooperative work upon these materials is, therefore, during cold weather. The associate referee has a supply of identical samples in storage and is desirous of obtaining more collaborators.

## RECOMMENDATIONS.

It is recommended—

(1) That the study of ash determination by both the direct and sulfate methods be continued.

(2) That a comparative study of methods for the determination of specific gravity and of total solids of molasses be undertaken.

---

[1] Presented by O. S. Keener.
[2] *J. Assoc. Official Agr. Chemists*, 1921, 4: 451.
[3] *Ibid.*, 444.

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## ANNOUNCEMENTS

Since publication of its *Journal* was begun it has been the hope of the Association of Official Agricultural Chemists that it might eventually be possible to use *The Journal* as a medium for the publication of research papers of a high grade, in addition to the reports of referees and other material included in the proceedings of the association.

The Board of Editors is pleased to announce that it is now ready to accept contributions of this kind for publication, since space in the forthcoming August and November numbers will be available for that purpose.

Unless otherwise instructed by the association, preference will be given by the editors to articles of special or general interest to agricultural chemists and, so far as space may require a discrimination, to papers of an analytical character or those dealing with methods of analysis.

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Occasional requests are being received for back numbers of *The Journal*, usually for the purpose of completing sets either for individuals or for libraries. The editors are unable to supply any numbers antedating the issue of November 15, 1919, or Number 2 of Volume III. It will be appreciated if subscribers to *The Journal* who may have numbers issued prior to November 15, 1919, which they are willing to dispose of, will notify us to this effect. For such of these numbers as may be required the full subscription price will be paid. Communications should be addressed to the Chairman of the Board of Editors, Box 290, Pennsylvania Avenue Station, Washington, D. C.

R. W. Balcom,
R. E. Doolittle,
C. B. Lipman,
R. B. Deemer.
*Board of Editors.*

WILLIAM FREAR, 1860—1922

# WILLIAM FREAR

William Frear was born in Reading, Pennsylvania, March 24, 1860, and passed away at his home at State College, Pennsylvania, January 7, 1922. The end came suddenly following a stroke of apoplexy. His wife, four children and a host of friends mourn his loss.

He was of Huguenot ancestry, the first member of the family, Hugo Frear, having settled in New Paltz, New York, in 1677. William Frear was the son of the Rev. George and Malvina (Rowland) Frear. His father was a Baptist minister at Reading and Norristown, where he attended the public schools. Later they moved to Lewisburg, Pennsylvania, where William Frear entered Bucknell University with the purpose of fitting himself to be a civil engineer. He, however, developed a taste for chemistry and the natural sciences and after graduation accepted a position as assistant in natural science at Bucknell University.

He received his Bachelor of Arts degree from Bucknell University in 1881. Later he pursued postgraduate work at Harvard University and at the Illinois Wesleyan University, and from the latter institution received, in 1883, the degree of Doctor of Philosophy. His work both in the public schools and in the universities was characterized by excellency of scholarship.

Dr. Frear began his life work, after graduation, as assistant chemist in the Bureau of Chemistry, U. S. Department of Agriculture, under Dr. H. W. Wiley, the newly appointed chief chemist, a position which he held until 1885, when he was appointed assistant professor of agricultural chemistry at the Pennsylvania State College. He, however, always kept in close touch with the U. S. Department of Agriculture, serving as special agent of the Department from 1900 until his death and as chairman of the Food Standards Committee. A year after his appointment at State College he was advanced to the rank of professor and served as professor of experimental agricultural chemistry and head of the department from 1908 until the time of his death. He was also vice-director of the Agricultural Experiment Station from 1887, two years after its organization, until the time of his death.

The esteem in which he was held for his scholarship, scientific attainments, broad vision, his ability to weigh and solve difficult problems, and his capacity for work, caused his services to be requisitioned not only in the college, but in the community, state and nation. For example, he was chemist of the State Board of Agriculture from 1888 until the Board was abolished in 1919; chemist in charge of analysis of fertilizers under the State Fertilizer Control from 1888 until 1919; chemist of the Pennsylvania State Department of Agriculture from 1895; chemist of the State Dairy and Food Commission from 1895; chemist of the State Cattle Food Control from 1902 until 1905; editor of Agricultural Science from 1892 to 1894; secretary of the Society for the Promotion of Agricultural Science from 1893 to 1895, and president of that organization in 1903.

While best known for his interest and work in connection with pure foods, food definitions, food standards and legislation, Dr. Frear was regarded as an authority on a wide range of subjects, such as soils, fertilizers, lime, feeds, tobacco culture and meteorology. The scope of his investigations is indicated by the number of papers on a great variety of subjects of which he was author or joint-author, amounting to a hundred or more bulletins and special manuscripts published by the Experiment Station and in scientific journals. The papers show careful scientific work and are expressed in language in keeping with his excellent and thorough literary training. In addition to his investigational work, he taught agricultural chemistry and various other lines of agriculture at State College for many years. One who knew him best says: "It was a great privilege to be a member of his classes. He was unusually careful in the presentation of material and most skilful in helping his students to analyze and grasp the problems involved".

On account of his wide range of information, so essential in legislation of this kind, and because of his clear thinking and exceptionally good judgment, he was often called into consultation when laws were being framed relating to foods, fertilizers, lime and other subjects of importance in agriculture. For the same reasons, his counsel was in demand in community matters, such as schools, churches, water system, street improvement, and indeed in every movement of importance to the welfare of the community, in which he always took an active interest.

He was an honored member of scientific societies, such as the American Chemical Society, the Academy of Political and Social Science, the Washington Academy of Sciences and the Association of Official Agricultural Chemists; and a highly esteemed member of several Greek letter fraternities. He was also a member of the Cosmos Club of Washington, and of Bellefonte Lodge No. 268, Free and Accepted Masons, of which he was master in 1907. He was also post commander of Constans Commandery of Knights Templar.

Such was Dr. Frear, scientist and scholar. His long, faithful and efficient service at State College, in his community, state and nation, and his conspicuous ability as a teacher, investigator, administrator, author and lecturer, challenge our highest admiration. But what of Frear the man? "A gentleman; a Christian gentleman. His personality was most admirable", so says Dr. Wiley, who has perhaps known him longest. "With all our admiration of Dr. Frear as an eminent scientist, we admire another side of the man still more; his splendid Christian character and fine human qualities that left an indelible impression upon the lives of all those who knew him, and upon thousands of people who counted him as their friend. He possessed to a remarkable degree the admirable qualities of kindness, cheerfulness, patience, charity and thoughtfulness. No one was ever associated with him in any capacity who was not helped in some way", is the testimony of one who was thrown in daily contact with Frear the man. This estimate of Frear the man is in perfect agreement

with the impression gained by the writer of this sketch during ten years' association with him in the activities of the Association of Official Agricultural Chemists. His conspicuous ability and admirable personality made him an outstanding figure at every meeting of the association.

It seems fitting in closing this tribute to the life and work of Dr. Frear to make special mention of his long, faithful and efficient service as a member of this association. According to the records, Dr. Frear first became identified with the Association of Official Agricultural Chemists at its fourth meeting, 1887. At the sixth meeting, 1889, he was made a member of the Executive Committee. At the eighth meeting, 1891, he made a very valuable report, "Abstracts of Various Methods of Determining Nitrogen", which no doubt led to the appointment, at the ninth meeting, 1892, of a Committee on Abstracts of Methods of Analysis, of which he was made chairman, and served as such for several years. He was vice-president at the thirteenth meeting, 1896, and president the following year, 1897. The following statement from his presidential address is worthy of quotation: "Primarily, the work of the association has been chiefly along the lines of importance to the official chemist. This must still be, to a large extent, true of the association's work. But it will fail of its opportunities and choose an ideal lower than it may properly select if its work be not pushed also, in large measure, along more distinctly scientific lines". In this address also he cited, in connection with his work on food adulteration, statistics showing the value of the food products of the country, amount of consumption and related subjects, and called especial attention to two ways in which this association could help the food control chemist: "(1) By a careful selection, accurate description, and test of methods fitted for the control examination of the various classes of adulterated food materials upon the market; (2) by taking steps to secure the establishment of standards of composition for pure food substances, just as druggists have done for drugs. * * * A similar work must be done for foods."

It is noteworthy that at this meeting a Committee on Food Standards was appointed, consisting of Messrs. Wiley, Weber, Scoville, Jenkins and Frear. Dr. Frear served as chairman of this committee during its entire existence. He made practically all of its reports. In 1901, on the resolution of Dr. Frear, this committee cooperated with the National State Dairy and Food Commissioners. Writing of his work in connection with these committees Dr. Wiley, who is of all men best qualified to speak, says: "He did the bulk of the clerical work and took care of the correspondence incident to the great work of establishing standards. I think his work was in that line, and I believe he remained chairman of the Standards Committee up until the time of his death. The actual volume of printed matter containing the standards adopted is small, but the data which were collected, the literature which was read, and the hearings which were held, if they could all be published would be most voluminous. It was Dr. Frear's duty and privilege to sift and sort, to condense

and prepare for action this large mass of material. He did this with care and industry and a success which are not at all recognized by the public at large. * * * He never became angry. He never rubbed a witness the wrong way. He was a discreet, cool and efficient presiding officer, and whatever the credit there be to be paid to the standards adopted, he should receive the greater part".

This committee functioned under Congressional authority until the passage of the Federal Food and Drugs Act in 1906. Later, in 1913, a new committee, the so-called Joint Committee of Definitions and Standards of the U. S. Department of Agriculture, was founded. Dr. Frear was again called upon for service. He was chairman of this committee at the time of his death.

Dr. Frear rarely if ever missed a meeting of the association from the time he became identified with it in 1887, until the time of his death. He always took a vital interest in all its activities and contributed freely and wholeheartedly of his time and talents in making them a success. He was an honored member of this association, having served on its Executive Committee, as vice-president, as president, as a member of numerous committees, and as a member of the Board of Editors of *This Journal* from 1920 until his death.

In the passing of Dr. William Frear the association has lost a most capable worker and a wise counsellor, and many of its members, a warm personal friend. In life it could well be said of him:

"Whom God illumines,
Dwells in undimmed day;
'Mid storm and night,
He treads a clear, sure way".

When the summons came he met death, of which he had no fear, "like one who wraps the drapery of his couch about him and lies down to pleasant dreams".

R. N. Brackett.

## FIRST DAY.

# MONDAY—AFTERNOON SESSION.

## REPORT ON FERTILIZERS.

By R. N. BRACKETT (Clemson Agricultural College, Clemson College, S. C.), *Referee.*

The work for 1921 followed the lines of former recommendations, and the results are contained in the reports of the different referees, together with their recommendations.

Nothing very startling developed this year. The nervous condition of the consumer about borax in fertilizers seems to have died a natural death, like so many other scares in the past; or perhaps confidence has been restored by the reappearance of foreign potash on our markets. Like so many ills, this borax scare served a good purpose, both in stimulating work and improving our method of approach—through general referees.

A paper, entitled "The Development of Accuracy in Fertilizer Analysis and Some Pitfalls in Methods"[1], by P. McG. Shuey, read before the Fertilizer Division of the American Chemical Society, may be worthy of consideration. It refers to the official volumetric method for the determination of phosphoric acid in fertilizers[2] and also to the details of the official method for determining nitrogen when nitrates are present in fertilizers[3]. The suggestion pertaining to the latter method is in complete accord with your referee's experience and practice.

J. H. Mitchell, who had planned to cooperate in the work on borax in fertilizers, took up with two of the senior students of Clemson College, as a thesis problem, a comparison of the Ross-Deemer, the Bartlett, and the Pope-Ross methods of analysis, and, with another student, the determination of nitrogen in nitrates in which the Devarda method was tried out. A few comments on the results of this work will be given by the associate referee.

Last year Arthur W. Clark, Agricultural Experiment Station, Geneva, N. Y., presented a paper before this association, entitled "A Method for the Determination of Phosphoric Acid"[4]. No recommendation was made for further investigation, but Mitchell took this also as a thesis problem. The results were not promising for the application of the method in routine work, though fairly accurate results may be obtained under the proper conditions of manipulation.

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[1] *Am. Fertilizer*, 1921, 55: 52.
[2] *Assoc. Official Agr. Chemists, Methods*, 1920, 3.
[3] *Ibid*, 7.
[4] *J. Assoc. Official Agr. Chemists*, 1921, 5: 103.

# REPORT ON THE DETERMINATION OF BORIC ACID IN FERTILIZERS AND FERTILIZER MATERIALS.

By WILLIAM H. ROSS (Bureau of Soils, Washington, D. C.), *Associate Referee.*

A summary of results obtained in a comparative study by eight collaborators of three quantitative methods for determining water-soluble boric acid as borax in fertilizers and fertilizer materials[1] was given last year. After the work was completed a modified distillation method which appeared to offer some advantages over other distillation methods was proposed by J. M. Bartlett[2] of the Maine Experiment Station. In accordance with the recommendations that the Ross-Deemer[3] method be adopted as a tentative method and that further work be done in comparing this method with the Bartlett distillation method, a collaborative study of the relative merits of the two methods in question was undertaken.

## PREPARATION OF SAMPLES FOR COLLABORATIVE WORK.

The samples submitted to the different collaborators consisted of three mixed fertilizer samples and three of potash salts. Sample No. 1 was a 5-8-5 fertilizer, the same as No. 3 of last year, and contained 0.1 per cent. of anhydrous borax. Sample No. 2 was the same as No. 1 with 0.5 per cent of borax. Sample No. 3 contained 1.15 per cent of borax and was prepared from a 5-10-0 fertilizer and a mixture of salts in imitation of crude Searles Lake potash. Samples Nos. 4, 5 and 6 were made of borax-free salts in imitation, respectively, of potash manure salts, commercial Chilean nitrate and the salts in the brine of Searles Lake. No borax was added to Sample No. 4; 0.5 per cent was added to No. 5; and 3.0 per cent was added to No. 6.

The borax used was prepared by adding a known amount of boric acid of a high degree of purity to a solution of an equivalent amount of pure sodium carbonate. The solution was then evaporated on a water bath, dried at 110°, weighed and ground to pass a 175-mesh sieve. Knowing the weight of the boric acid taken and of the product finally obtained, it could then be calculated how much of the product would have to be added to a fertilizer to give a borax content equivalent to any desired percentage of anhydrous borax. As a check against any possible loss of borax in the course of its preparation, weighed portions of the recovered product and of the boric acid from which it was prepared were titrated against the same standard alkali.

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[1] *J. Assoc. Official Agr. Chemists*, 1921, 5: 83.
[2] *Ibid.*, 1921, 5: 90.
[3] *Ibid.*, 327; *Am. Fertilizer*, 1920, 52: 62.

## INSTRUCTIONS TO COLLABORATORS.

A detailed account of each method was forwarded with the samples to each collaborator, and it was requested that each analyst submit a report on completing the work indicating the method which, in his judgment, gave most accurate results and which was considered most rapid for each sample analyzed, if applied to routine analysis. The reports received from ten collaborators are summarized in Table 1.

TABLE 1.

*Determination of boric acid in mixed fertilizers and fertilizer materials.*

| ANALYST | BORIC ACID EXPRESSED AS ANHYDROUS BORAX | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Sample No. 1 Borax, 0.10% | | Sample No. 2 Borax, 0.50% | | Sample No. 3 Borax, 1.15% | | Sample No. 4 Borax, none | | Sample No. 5 Borax, 0.50% | | Sample No. 6 Borax, 3.00% | |
| | Ross-Deemer | Bartlett | Ross-Deemer | Bartlett | Ross-Deemer | Bartlett | Ross-Deemer | Bartlett | Ross-Deemer | Bartlett | Ross-Deemer | Bartlett |
| | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* |
| C. A. Butt, International Agricultural Corporation Atlanta, Ga | 0.09 | 0.07 | 0.45 | 0.43 | 1.31 | 1.15 | 0.00 | 0.01 | 0.48 | 0.48 | 2.85 | 2.75 |
| L. W. Willis, Agricultural Experiment Station, New Brunswick, N. J. | 0.09 | 0.10 | 0.36 | 0.45 | 1.00 | 1.14 | 0.06 | .... | 0.50 | .... | 2.84 | ... |
| H. B. McDonnell, Agricultural Experiment Station, College Park, Md. | 0.10 | 0.14 | 0.40 | 0.50 | 0.91 | 1.06 | 0.04 | 0.05 | 0.57 | 0.53 | 2.95 | 2.93 |
| R. M. Jones, Bureau of Soils, Washington, D. C. | 0.12 | 0.19 | 0.46 | 0.46 | 1.10 | 1.26 | 0.00 | 0.00 | 0.49 | 0.51 | 2.88 | 2.67 |
| William Hazen, Bureau of Soils, Washington, D.C. | 0.13 | 0.10 | 0.48 | 0.43 | 1.15 | 1.13 | 0.01 | 0.02 | 0.50 | 0.47 | 3.00 | 2.77 |
| Millard G. Moore, Agricultural Experiment Station, Geneva, N. Y. | 0.14 | 0.09 | 0.44 | 0.42 | 0.88 | 1.06 | 0.00 | 0.00 | 0.48 | 0.44 | 2.84 | 2.65 |
| Ethel Schram, Armour & Co., Chicago, Ill. | 0.11 | 0.06 | 0.44 | 0.57 | 1.12 | 0.29 | 0.06 | 0.01 | 0.47 | 0.50 | 2.86 | 2.90 |
| E. R. Tobey, Agricultural Experiment Station, Orono, Me. | 0.09 | 0.09 | 0.40 | 0.39 | 1.05 | 0.99 | 0.00 | 0.00 | 0.53 | 0.44 | 2.81 | 2.67 |
| C. H. White, Agricultural Experiment Station, Orono, Me. | 0.07 | 0.09 | 0.29 | 0.42 | 0.80 | 1.07 | 0.00 | 0.00 | 0.49 | 0.45 | 3.01 | 2.57 |
| W. C. Weltman, Agricultural Experiment Station, Orono, Me. | 0.07 | 0.06 | 0.43 | 0.40 | 1.02 | 1.02 | 0.00 | 0.00 | 0.50 | 0.61 | 2.81 | 2.88 |
| Average | 0.10 | 0.10 | 0.42 | 0.45 | 1.03 | 1.02 | 0.02 | 0.01 | 0.50 | 0.49 | 2.89 | 2.75 |

## DISCUSSION.

The averages of the results reported by the different collaborators show that so far as accuracy is concerned there is no choice between the

two methods. In the matter of rapidity it appears that neither method is superior or equally suitable for all classes of materials. The Bartlett method is more convenient for the analysis of materials high in soluble phosphates or organic matter relative to the boric acid, while the Ross-Deemer method is preferred for materials which are relatively low in these constituents. A summary of the reports made by the different collaborators on the relative rapidity of the two methods is given in Table 2. One of the collaborators made no comments on either method; two expressed no preference in the case of Sample No. 4; and one made no choice in the case of Sample No. 5.

TABLE 2.

*Summary of reports of collaborators on relative rapidity of methods.*

| SAMPLE NO. | NUMBER OF COLLABORATORS REPORTING IN FAVOR OF METHOD OF— | |
|---|---|---|
| | Ross-Deemer | Bartlett |
| 1 | 1 | 8 |
| 2 | 2 | 7 |
| 3 | 2 | 7 |
| 4 | 6 | 1 |
| 5 | 8 | 0 |
| 6 | 8 | 1 |

In commenting on the combined reports of the different collaborators, Bartlett called attention to the advantage of a chemist having a choice of two methods of entirely different procedure but of equal accuracy, and of being able to check one against the other on special occasions, as when testimony is to be given in court.

It may be emphasized, as was pointed out by Butt, that the Bartlett method is likely to give acid-soluble boric acid while the Ross-Deemer method gives only water-soluble boric acid. The samples submitted to the collaborators contained borax only, and the results with both methods therefore agree, but this may not be true in the case of samples containing both soluble and insoluble boric acid, unless the procedure of analysis is modified to give water-soluble or acid-soluble boric acid, as the case may require.

## RECOMMENDATIONS.

It is recommended—

(1) That the Bartlett method be adopted as a tentative method for the determination of boric acid in fertilizers and fertilizer materials on account of its special adaptation to the analysis of samples which are relatively high in soluble phosphates or organic matter.

(2) That the Ross-Deemer method be retained as a tentative method for the determination of water-soluble boric acid in fertilizers and ferti-

lizer materials on account of its special adaptation to the analysis of samples which are low in soluble phosphates and organic matter relative to the boric acid.

(3) That further work be done on both methods recommended as tentative to determine the effect of insoluble boric acid and to study any modifications necessary to make both methods applicable to the determination of water-soluble, acid-soluble or total boric acid, as the case may require.

## REPORT ON THE PREPARATION OF A NEUTRAL SOLUTION OF AMMONIUM CITRATE.

By C. S. ROBINSON (Agricultural Experiment Station, E. Lansing, Mich.), *Associate Referee.*

In accordance with the recommendation of the association that the colorimetric method "be given further study, with collaboration, with a view to adoption in 1921", the associate referee asked each collaborator

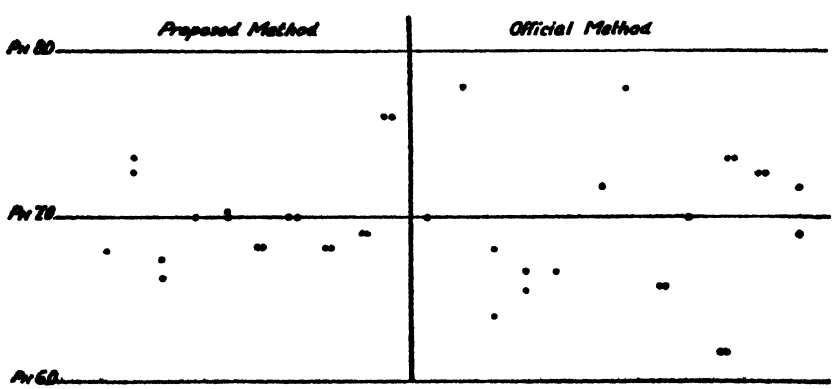
TABLE 1.

*Reaction and composition of ammonium citrate solutions.*

| COLLABORATOR | OFFICIAL METHOD | | | PROPOSED METHOD | | |
|---|---|---|---|---|---|---|
| | pH | Ratio of ammonia to anhydrous citric acid | | pH | Ratio of ammonia to anhydrous citric acid | |
| | | Referee | Collaborator | | Referee | Collaborator |
| R. F. Gardiner, Bureau of Soils, Washington, D. C. | 7.0 | 1:3.740 | ..... | 6.8 | 1:3.760 | ..... |
| W. H. Strowd, Department of Agriculture, Madison, Wis. | 7.8 | 1:3.680 | ..... | .... | ..... | ..... |
| R. D. Caldwell, Armour Fertilizer Works, Atlanta, Ga. | 6.4 | 1:3.946 | 1:3.841 | 7.3 | 1:3.749 | 1:3.694 |
| | 6.8 | 1:3.805 | 1:3.709 | 7.4 | 1:3.756 | 1:3.690 |
| E. E. Vanatta, Agricultural Experiment Station, Columbia, Mo. | 6.6+ | 1:3.838 | 1:3.690 | 6.6 | 1:3.859 | 1:3.620 |
| | 6.7 | 1:3.797 | 1:3.580 | 6.7+ | 1:3.805 | 1:3.590 |
| G. Hart, Agricultural Department, Tallahassee, Fla. | 6.7 | 1:3.810 | ..... | ... | ..... | ..... |
| P. H. Wessels, Agricultural Experiment Station, Kingston, R. I. | 7.2 | 1:3.763 | ..... | 7.0 | 1:3.757 | ..... |
| A. P. Kerr, Agricultural Experiment Station, Baton Rouge, La. | 7.8 | 1:3.767 | 1:3.787 | 7.0 | 1:3.814 | 1:3.797 |
| | | | | 7.0+ | 1:3.809 | 1:3.797 |
| E. G. Proulx, State Chemist, Lafayette, Ind. | 6.6 | 1:3.888 | ..... | 6.8+ | 1:3.835 | ..... |
| | 6.6 | 1:3.895 | ..... | 6.8+ | 1:3.831 | ..... |
| W. D. Richardson, Swift & Co., Chicago, Ill. | 7.2 | 1:3.813 | 1:3.777 | 7.0− | 1:3.826 | 1:3.759 |
| | 7.0 | 1:3.823 | 1:3.776 | 7.0− | 1:3.819 | 1:3.762 |
| Armour & Co., New Orleans, La. | 6.2 | 1:4.005 | 1:3.665 | 6.8 | 1:3.836 | 1:3.837 |
| | 6.2 | 1:4.007 | 1:3.732 | 6.8 | 1:3.831 | 1:3.866 |
| J.W. Kellogg, Department of Agriculture, Harrisburg, Pa. | 7.4− | 1:3.776 | ..... | 6.9 | 1:3.836 | ..... |
| | 7.4− | 1:3.767 | ..... | 6.9 | 1:3.825 | ..... |
| J. H. Parkins, F. S. Royster Guano Co., Norfolk, Va. | 7.3 | 1:3.742 | ..... | ..... | ..... | ..... |
| | 7.3 | 1:3.735 | ..... | ..... | ..... | ..... |
| L. F. Schmelzer, Armour & Co., Chrome, N. J. | 6.9 | 1:3.799 | 1:3.779 | 7.6 | 1:3.748 | 1:3.729 |
| | 7.2 | 1:3.744 | 1:3.759 | 7.6 | 1:3.743 | 1:3.809 |

to submit samples of four solutions. Two of these were to be prepared by an official method and two by the proposed colorimetric method. Thirteen chemists sent in solutions prepared by one or the other of the official methods, and eleven submitted samples made up by the proposed method. One of the sets made by the proposed method was discarded because the proper standard had not been used. One set of solutions neutralized to litmus paper was also received. The reactions of the solutions were measured colorimetrically against standards which had been checked electrometrically, and the ratios of ammonia to citric acid were determined by the formaldehyde titration method. The results are shown in the table and chart.

It is at once apparent that the colorimetric method gives, as a rule, solutions more nearly neutral than do the official methods. Not only are a greater number of truly neutral solutions prepared by the new method than by the official methods, but the range of variation from the neutral point is much narrower. Of the twenty-one solutions prepared by the official methods only seven (33 per cent) come within a range of pH 6.8—7.2 as compared with a total of twelve (67 per cent) of those prepared by the new method. Only three solutions (14 per cent) of those prepared by the official methods were within one point of neutrality, while seven (39 per cent) of those prepared by the colorimetric method were actually neutral within this range of error.




COMPARISON OF REACTIONS OF AMMONIUM CITRATE SOLUTIONS PREPARED BY THE OFFICIAL AND PROPOSED METHODS.

Unfortunately, complete data on the particular official method used by each collaborator are not available so that no comparison can be made between the results with corallin[1] and with the alcoholic calcium chloride method[2]. However, as one analyst only reported the use of

[1] *Assoc. Official Agr. Chemists, Methods*, 1920, 4, (1).
[2] *Ibid.*, (2).

the latter, while several stated that they had used the former, it may be assumed that but few laboratories use the longer and more complicated procedure, which, the writer is convinced, has nothing to recommend its retention as an official method.

One point was brought to the attention of the writer in connection with this work which should be considered by the association before the subject is closed. This is the method of analysis to be used in checking up the composition of ammonium citrate solutions. Several modifications are used, some of which are not reliable. This accounts for some of the differences in the results of the analyses by the collaborators and the referee. Changes in the composition of the solutions owing to the loss of ammonia or solution of alkali from the glass containers are factors which influence the results in some cases.

The results of a study of this subject have been published elsewhere[1] as the writer considered that they dealt with a different phase of the citrate question than that covered by the recommendation of the association.

## RECOMMENDATIONS.

It is recommended—

(1) That a neutral solution of ammonium citrate be considered as one in which the ratio of ammonia to anhydrous citric acid is as $1:3.794 \pm 0.02$, and that it shall have a reaction corresponding to a pH of $7.0 \pm 0.2$.

(2) That that section of the official methods dealing with the preparation of neutral solutions of ammonium citrate[2] be changed to read as follows:

**12** REAGENTS.

In addition to the reagents described under **4** and **7** prepare ammonium citrate solution by the following method:

*Ammonium citrate solution.*—For every liter of solution required dissolve 172.00 grams of anhydrous or 188.13 grams of crystallized citric acid in approximately 700 cc. of water; nearly neutralize with ammonium hydroxide; cool; measure the volume of solution or make it up to a convenient volume, taking care to keep the density above 1.09; make exactly neutral, testing as follows: With a pipet transfer 5 cc. of the citrate solution to a test tube (preferably 7x⅞ inches) and dilute to 20 cc. with distilled water. Add from a dropping bottle 5 drops of a 0.08% solution of phenol red indicator. From a buret run in standard ammonia solution until the color approximates that of a standard buffer solution having a pH of 7.0 (prepared by mixing 50 cc. of 0.2M dihydrogen potassium phosphate solution and 29.63 cc. of 0.2N sodium hydroxide solution and making up to 200 cc.) contained in a similar test tube and with the same concentration of indicator. Complete the process by carefully adding the standard ammonia solution in small amounts and comparing the colors in a comparator. From the amount of ammonia solution required to produce in the sample a color which exactly matches that of the standard, calculate the amount required to neutralize the rest of the solution.

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[1] *J. Ind. Eng. Chem.*, 1922, **14**: 429.
[2] *Assoc. Official Agr. Chemists, Methods*, 1920, 4.

Add this calculated amount of ammonia to the original solution and check its reaction against that of the neutral standard, using the technique described above. If the colors match, dilute the solution to a density of 1.09 at 20°.

Finally check the composition of the solution by determining the ratio of ammonia to citric acid by analysis.

(3) That the methods of analysis of ammonium citrate solutions be studied.

## SOME EXPERIENCES WITH THE ALKALINE PERMANGANATE METHOD[1].

By C. S. ROBINSON and O. B. WINTERS (Agricultural Experiment Station, E. Lansing, Mich.).

Realizing that the alkaline permanganate method[2] for the measurement of the quality of organic nitrogen in fertilizers had certain inherent faults, the writers attempted some time ago so to modify it as to overcome these defects. After spending considerable time, it was concluded that no progress could be made by simple hit-and-miss trials, but that some fundamental knowledge of the actual chemical factors involved in availability and its measurement must first be gained. Consequently, attempts to develop a modification of the permanganate method were discontinued, and a study was made of the actual effect of the procedure ordinarily followed upon chemical substances of more or less definite constitution. The results of the preliminary work are reported merely to have them on record for the benefit of anyone who may be tempted to try the same line of attack. It is only fair to state in this connection that similar work had been done by the originator of the method, C. H. Jones, and that his conclusions were verified by the present writers.

It is probably a safe assumption that the organic compounds of greatest importance in fertilizers are of protein origin. From the writers' knowledge of the chemistry of such substances the action of alkaline permanganate solution upon them may be divided into two steps. The first, the action of the alkali, i. e. the hydroxyl ions, will result in a hydrolytic cleavage of the protein compounds producing ultimately amino acids and acid amides. An analogous action takes place in the soil under the influence of bacterial and chemical agents. Another result of the alkali in the reagent employed is the decomposition of a portion of the acid amides present with the liberation of ammonia. These compounds, i. e. the acid amides, furnish practically all of the ammonia liberated directly by the action of the alkali, only

[1] Journal Article No. 21 from the Chemical Laboratory of the Michigan Agricultural College Experiment Station. Published by permission of the Director of the Experiment Station.

[2] *Assoc. Official Agricultural Chemists, Methods*, 1920, 11.

a small portion being given off by the other compounds as the result of the action of alkali alone. In short, the alkali of the permanganate reagent breaks down the complex organic nitrogenous compounds into simpler ones, i. e. amino acids and acid amides, and converts a portion of the latter into ammonia.

The second step in the process (which, of course, goes on simultaneously with the first) is the conversion of a fraction of the amino-acid nitrogen formed by the alkali into ammonia. The ultimate effect then of both alkali and permanganate is the production of ammonia which is used as a measure of the value of the material under examination.

No doubt it is possible with the alkaline permanganate reagent to measure the total amounts of acid amide and amino-acid nitrogen obtainable from any substance if treatment is continued long enough. All of this nitrogen can be used by plants for food purposes, in some cases directly and in others indirectly after conversion into ammonia. Any nitrogen which can be so converted must be considered as being potentially available. Its measurement presents no problem. The difficulty lies in the determination of that portion of this potentially available fraction which will be made available during a growing season.

The simplest assumption is that this is determined by the rate at which potentially available nitrogen is converted into amino acids, acid amides and ammonia. This rate would be different for different materials whose availability would vary directly with it. If this assumption is justified, then it should be possible to find some set of laboratory conditions under which organic nitrogenous materials would be decomposed at the same *relative* rates as they are in the soil. A method based upon this procedure would involve the measurement of a definite property common to all of the materials to which it would be applied. This the present method does not do, although it is only under such conditions that results can be obtained which are properly comparable.

Two values offer themselves naturally for consideration: (1) The time required for the ammonification of a selected fraction of the potentially available portion; and (2) the fraction of the nitrogen ammonified in a given time. The value of the second factor is that which the present method attempts to measure. In some cases it actually does this while in others it does not. With materials which do not decolorize the permanganate the results obtained show the nitrogen ammonified during the time required for the determination. With cottonseed meal, peat and other substances which do decolorize the solution the meaning of the results is quite different since the ammonia produced by the second step of the process, as outlined above, is materially reduced. With such materials the principal effect is that of the alkali, the permanganate being removed from the sphere of action early in the process. Hence, in effect, the results of two entirely different reagents are compared

when an attempt is made to compare the figures obtained with these two classes of materials, i. e. those which do not and those which do decolorize the permanganate. In reality, values obtained for materials of the latter class are low, not because of their poor quality, but because of the manner in which they are treated.

If conditions are so altered that the permanganate is not decolorized, the second group of substances gives an entirely different picture. In Table 1 are shown the values for the active insoluble nitrogen in cottonseed meal and peat, (1) as obtained by the official method, and (2) by substituting for the samples containing 50 milligrams of nitrogen, ones so small that the permanganate was not decolorized.

TABLE 1.

*Comparison of the action of permanganate on varying samples of peat and cottonseed meal.*

| | TOTAL NITROGEN | AMINO NITROGEN + AMIDE NITROGEN |
|---|---|---|
| | *per cent* | *per cent* |
| Cottonseed meal—full sample | 45.00 | ..... |
| Cottonseed meal—half sample | 70.60 | 68.42 |
| Peat—full sample | 22.32 | ..... |
| Peat—one-eighth sample | 66.00 | 59.79 |

It is evident that when judged by the same standards the differences between high- and low-grade nitrogen are greatly diminished.

A second point of fundamental importance in the use of the alkaline permanganate method is the fact that the concentration of the reagents, especially the alkali, is constantly increasing, until, at the end of the distillation, the speed of any reaction not already completed is presumably high. Thus any variation in time will produce a corresponding variation in results. Yet with the tendency on the part of the sample to foam badly, the digestion and distillation periods are frequently lengthened far beyond the recommended 90 minutes. The amounts of nitrogen in the last portions of the distillates from several materials are shown in Table 2.

As it is difficult to gage the distillate closer than 10–15 cc. with the ordinary sized flasks used in this procedure, i. e. 500 cc. Kjeldahl and 300 cc. receiving flasks, the above figures represent possible errors in every-day practice.

From the above considerations it seemed advisable to modify the method with respect to the following points:

(1) The size sample selected should be such that an excess of permanganate would always be present.

(2) The final concentration of reagents should be such that reason-

TABLE 2.

*Ammonia given off in the last portions of the distillate in the alkaline permanganate method.*

| | Sample* No. 23 | Sample* No. 24 | Sample* No. 28 | Sample† No. 43 | Sample‡ No. 45 |
|---|---|---|---|---|---|
| | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* |
| Total nitrogen | 14.01 | 14.05 | 2.76 | 2.48 | 3.10 |
| Insoluble nitrogen | 12.97 | 13.25 | 2.52 | 1.66 | 1.31 |
| Insoluble nitrogen (Per cent of total nitrogen) | 92.57 | 94.30 | 91.30 | 66.93 | 42.26 |
| Active insoluble nitrogen | 9.93 | 10.53 | 0.92 | 1.11 | 0.52 |
| Active insoluble nitrogen (Per cent of total nitrogen) | 70.87 | 74.96 | 33.34 | 44.75 | 16.77 |
| Active insoluble nitrogen (Per cent of insoluble nitrogen) | 76.56 | 79.47 | 36.51 | 66.86 | 39.69 |
| Nitrogen in distillate | 0.62 | 0.40 | 0.13 | 0.07 | 0.06 |
| Nitrogen in distillate (Per cent of total nitrogen) | 4.43 | 2.85 | 4.71 | 2.82 | 1.93 |
| Nitrogen in distillate (Per cent of insoluble nitrogen) | 4.78 | 3.02 | 5.16 | 4.22 | 4.58 |

*Amount of distillate, 15 cc.
†Amount of distillate, 12 cc.
‡Amount of distillate, 10 cc.

able variations in the time of distillation and the amount of distillate would not produce significant variations in the results.

With this in view permanganate solutions of various strengths were used; the processes were carried out for different lengths of time and different sized samples were taken. No set of conditions was found, however, that seemed to give any promise of success. The following procedure and the results obtained with it are typical:

Weigh a ½- (or ¼- where the material is high in nitrogen) gram sample into a funnel containing a 12.5 cm. filter paper and wash with 250 cc. of distilled water. Transfer the insoluble residue to a 500 cc. Kjeldahl flask. Add 300 cc. of 0.5N alkaline permanganate solution (containing 15.8 grams of potassium permanganate and 20 grams of sodium hydroxide per liter). Heat to boiling in about 20 minutes, digest 1 hour and distil off 150 cc. in 45 minutes.

It was found that most of the ammonia came over in the first 150 cc. That in the second 150 cc. came over slowly and hence a small variation in the conditions of distillation produced but small variations in the results.

The results show that, as was pointed out previously, changes in the relative amounts of sample and solution tend to exert a general leveling effect on the values found, diminishing the range of differences between high- and low-grade materials.

The authors' experiences led to two conclusions regarding the future development of methods for determining the availability of organic nitrogen: (1) That any new method must be built upon an entirely new foundation for which more fundamental chemical knowledge of the factors involved in availability is necessary; and (2) that it is highly

TABLE 3.

*Comparative results with official and modified alkaline permanganate methods.*

| Sample No. | INSOLUBLE NITROGEN, ACTIVE FORM | | Sample No. | INSOLUBLE NITROGEN, ACTIVE FORM | |
|---|---|---|---|---|---|
| | Official Method | Modified Method | | Official Method | Modified Method |
| | *per cent* | *per cent* | | *per cent* | *per cent* |
| 28 | 34.52 | 53.17 | 44 | 54.90 | 59.31 |
| 27 | 35.16 | 50.64 | 16 | 57.88 | 57.18 |
| 45 | 35.88 | 57.25 | 36 | 58.22 | 57.54 |
| 21 | 44.08 | 44.78 | 46 | 59.94 | 61.62 |
| 19 | 46.56 | 57.20 | 43 | 62.05 | 49.40 |
| 31 | 48.99 | 47.91 | 35 | 65.97 | 55.97 |
| 29 | 51.72 | 49.60 | 32 | 67.59 | 55.02 |
| 30 | 54.23 | 53.52 | 24 | 69.21 | 58.11 |

desirable that more systematic vegetation experiments be carried on, thus giving more reliable standards upon which to base conclusions regarding the values of any methods proposed. At the present time too few data on the relative actual values of various types of organic materials are available to make it safe to say that one method does and another does not give a fair estimate of their relative grades.

COMPARATIVE RESULTS WITH OFFICIAL AND MODIFIED ALKALINE PERMANGANATE METHODS.

## REPORT ON NITROGEN.

By I. K. PHELPS (Bureau of Chemistry, Washington, D. C.), *Associate Referee on Nitrogen in Fertilizers.*

A recommendation made by the association in 1920 directed the associate referee to make a study of the Devarda alloy method as applied to the determination of nitrate nitrogen in potassium and sodium nitrate. Owing to the pressure of other work, one sample only of carefully prepared potassium nitrate with a sufficient amount of Devarda alloy for conducting the work was sent to thirty chemists who had signified their willingness to cooperate. Six reports were received.

## INSTRUCTIONS TO COLLABORATORS.

### REAGENTS.

(a) *Devarda alloy, specially prepared.*

(b) *0.2N standardized acid.*

(c) *0.1N standardized alkali.*

(d) *Methyl red indicator.*—Dissolve 0.02 gram in 100 cc. of hot water (10 drops for each titration).

### DETERMINATION.

Into an 800 cc. Kjeldahl flask, measure 300 cc. of water, 3 grams of alloy and 3–5 cc. of sodium hydroxide solution (sp. gr. 1.453). Connect the flask at once with a Kjeldahl distilling apparatus fitted with a Davisson scrubber[1] or other suitable scrubbing bulb, preferably of Pyrex glass, into which 20–30 cc. of water have been drawn, and distil. During the process of distillation the tip of the condenser should always extend beneath the surface of the standard acid soluton, and the reducton of the nitrate should be carried on synchronously with the distillation. Regulate the boiling so that approximately 250 cc. of the distillate will be collected in the required time for each series of experiments. When the distillation is half completed it is recommended that part of the solution in the scrubber be sucked back into the distillation flask, allowing 10–20 cc. to remain in the bulb, this being accomplished by removing the flame or holding the flask out of place and applying a damp cloth. This removes the danger of the liquid in the bulb splashing over into the condenser, and by reducing the volume of liquid in the bulb, facilitates the passing of the last traces of ammonia into the receiving flask.

### EXPERIMENTS.

I. Three sets of blanks: (A) ¾ of an hour, (B) 1 hour and (C) 1¼ hours. First connect the flasks to the condenser by means of scrubbing bulb but omit sample, start the reduction, distil slowly and turn the flames up after 10 minutes so that 250 cc. of distillate are collected in Set A in ¾ of an hour, Set B in 1 hour and Set C in 1¼ hours.

II.—Dissolve exactly 4 grams of potassium nitrate in distilled water and dilute at standard temperature to 500 cc. in a volumetric flask. Mix thoroughly and transfer 25 cc. portions of this solution to the 800 cc. Kjeldahl flasks by means of an accurate pipet. With this sample repeat all the steps under Procedure I.

III.—Dissolve 10 grams of potassium nitrate in 500 cc. of water as above and take 25 cc. portions of this solution. Repeat as in Procedure II.

IV.—Repeat six of Procedure III, but bring sample to boiling as rapidly as possible instead of heating slowly for 10 minutes. Note time required in each case to collect the required amount of distillate.

All experiments should be made in triplicate and the results recorded in the enclosed table. It is suggested that Pyrex glass apparatus be used throughout; if, however, other glass is used, please note this fact; also include a measurement of the size of perforation in distilling shelf (3 inch hole is recommended) and the distance of the top of the burner from the flask; also note whether or not at any time during the distillation water was absent from the scrubbing bulb.

A. L. Prince and B. F. Robertson, for their own satisfaction, made further experiments other than those included in the instructions sent

[1] *J. Ind. Eng. Chem.*, 1919, 11: 465

out, using the Hopkins distilling bulb in place of the Davisson scrubber. They found that the results did not vary from the theoretical value any more than those which were conducted with the Davisson scrubber. These few results, however, are not included in the table since they are insufficient to be conclusive.

TABLE 1.

*Nitrogen in pure potassium nitrate.*

| ANALYST | TIME | WEIGHT OF POTASSIUM NITRATE | WEIGHT OF NITROGEN | BLANK | CORRECTED WEIGHT OF NITROGEN | NITROGEN |
|---|---|---|---|---|---|---|
| | *minutes* | *gram* | *gram* | *gram* | *gram* | *per cent* |
| A. L. Prince, Agricultural Experiment Station, New Brunswick, N. J. | 45 | 0.2 | 0.02766 | 0.00014 | 0.02752 | 13.76 |
| | 60 | 0.2 | 0.02772 | 0.00008 | 0.02764 | 13.82 |
| | 75 | 0.2 | 0.02779 | 0.00014 | 0.02765 | 13.83 |
| | 45 | 0.5 | 0.06909 | 0.00014 | 0.06895 | 13.79 |
| | 60 | 0.5 | 0.06939 | 0.00008 | 0.06931 | 13.86 |
| | 75 | 0.5 | 0.06900 | 0.00014 | 0.06886 | 13.77 |
| T. L. Roettger, Southern Cottonseed Oil Co., Savannah, Ga. | 45 | 0.2 | 0.02803 | 0.00030 | 0.02773 | 13.87 |
| | 60 | 0.2 | 0.02806 | 0.00021 | 0.02785 | 13.93 |
| | 75 | 0.2 | 0.02807 | 0.00028 | 0.02779 | 13.90 |
| | 45 | 0.5 | 0.06978 | 0.00030 | 0.06948 | 13.90 |
| | 60 | 0.5 | 0.06975 | 0.00021 | 0.06954 | 13.91 |
| | 75 | 0.5 | 0.06923 | 0.00028 | 0.06895 | 13.79 |
| W. D. Richardson, Swift & Co., Union Stock Yards, Chicago, Ill. | 45 | 0.2 | 0.02754 | 0.00023 | 0.02731 | 13.66 |
| | 60 | 0.2 | 0.02759 | 0.00013 | 0.02746 | 13.73 |
| | 75 | 0.2 | 0.02759 | 0.00011 | 0.02748 | 13.74 |
| | 45 | 0.5 | 0.06888 | 0.00023 | 0.06865 | 13.73 |
| | 60 | 0.5 | 0.06908 | 0.00013 | 0.06895 | 13.79 |
| | 75 | 0.5 | 0.06912 | 0.00011 | 0.06901 | 13.80 |
| Ethel Schram, Armour & Co., Union Stock Yards, Chicago, Ill. | 45 | 0.2 | 0.02775 | 0.00017 | 0.02758 | 13.79 |
| | 60 | 0.2 | 0.02799 | 0.00023 | 0.02776 | 13.88 |
| | 75 | 0.2 | 0.02784 | 0.00032 | 0.02752 | 13.76 |
| | 45 | 0.5 | 0.06954 | 0.00017 | 0.06937 | 13.87 |
| | 60 | 0.5 | 0.06951 | 0.00023 | 0.06928 | 13.86 |
| | 75 | 0.5 | 0.06972 | 0.00032 | 0.06940 | 13.88 |
| G. J. Kuhlmann, Jr., State Department Agriculture, Harrisburg, Pa. | 45 | 0.2 | 0.02710 | 0.00060 | 0.02650 | 13.25 |
| | 60 | 0.2 | 0.02727 | 0.00030 | 0.02697 | 13.49 |
| | 75 | 0.2 | 0.02727 | 0.00025 | 0.02702 | 13.51 |
| | 45 | 0.5 | 0.06883 | 0.00060 | 0.06823 | 13.65 |
| | 60 | 0.5 | 0.06843 | 0.00030 | 0.06813 | 13.63 |
| | 75 | 0.5 | 0.06823 | 0.00025 | 0.06798 | 13.60 |
| B. F. Robertson, Clemson Agricultural College, Clemson College, S. C. | 45 | 0.2 | 0.02490 | 0.00020 | 0.02470 | 12.35 |
| | 60 | 0.2 | 0.02673 | 0.00023 | 0.02650 | 13.25 |
| | 75 | 0.2 | 0.02723 | 0.00030 | 0.02693 | 13.47 |
| | 45 | 0.5 | 0.05520 | 0.00020 | 0.05500 | 11.00 |
| | 60 | 0.5 | 0.06425 | 0.00023 | 0.06402 | 12.80 |
| | 75 | 0.5 | 0.06713 | 0.00030 | 0.06683 | 13.37 |
| L. J. Jenkins and J. F. Ellis, Bureau of Chemistry, Washington, D. C. | 45 | 0.2 | 0.02774 | 0.00021 | 0.02753 | 13.77 |
| | 60 | 0.2 | 0.02776 | 0.00029 | 0.02747 | 13.74 |
| | 75 | 0.2 | 0.02786 | 0.00031 | 0.02755 | 13.78 |
| | 45 | 0.5 | 0.06944 | 0.00021 | 0.06923 | 13.85 |
| | 60 | 0.5 | 0.06942 | 0.00029 | 0.06913 | 13.83 |
| | 75 | 0.5 | 0.06935 | 0.00031 | 0.06904 | 13.81 |

*Comment by Robertson.*—Some of the low nitrogen results were due to the scrubber retarding the distillation if the time was less than 1 hour 30 minutes. The time given in the directions was not enough. If the distillation was continued until 50 cc. or less remained in the flask, the results very closely approached the theoretical. When the Hopkins bulb was used better results were obtained in the specified time. From work done with the scrubber, it was concluded (1) that it is not as accurate as the Hopkins bulb; (2) that it is a hindrance to rapid work; (3) that the method has no advantage over the regular Kjeldahl method, and (4) that it is not as accurate for regular routine work.

The experiments in Table 2 were conducted in order to determine whether or not any ammonia was lost by rapid reduction and distillation. Five out of seven of the reports indicate that no appreciable amount of ammonia is lost through the rapid reduction and distillation of the nitrate, while two reports show an appreciable loss. Here again it is believed the number of reports are insufficient to determine whether or not there is a loss of ammonia.

TABLE 2.

*Loss of ammonia by rapid reduction and distillation.*

| ANALYST | TIME | WEIGHT OF POTASSIUM NITRATE | NUMBER OF DETERMINATIONS | AVERAGE WEIGHT OF NITROGEN |
|---|---|---|---|---|
| | *minutes* | *gram* | | *gram* |
| A. L. Prince | 31 | 0.5 | 2 | 0.06913 |
| T. L. Roettger | 35 | 0.5 | 5 | 0.06440 |
| W. D. Richardson | 34 | 0.5 | 5 | 0.06911 |
| Ethel Schram | 52 | 0.5 | 5 | 0.06958 |
| G. J. Kuhlmann, Jr. | 28 | 0.5 | 5 | 0.06830 |
| B. F. Robertson | 75 | 0.5 | 6 | 0.06566 |
| Bureau of Chemistry | 33 | 0.5 | 6 | 0.06925 |

## CONCLUSION.

It is not possible to derive any definite conclusion from the figures representing the work of so few analysts. Six of the seven results reported, however, especially those in which the reduction and distillation were conducted for a period of one hour or more, agree sufficiently to warrant further investigation of this method for the determination of nitrate nitrogen. The number of sets in agreement indicate that the method is reasonably free from source of error and gives fairly uniform results in the hands of different analysts.

## RECOMMENDATIONS.

It is recommended—

(1) That the association continue the study of the Devarda method.

(2) That a comparison of results be made with the suggested modified Kjeldahl-Gunning method, by H. C. Moore[1], for the determination of nitrate nitrogen in nitrates and fertilizers.

[1] *J. Ind. Eng. Chem.*, 1920, 12: 669.

# THE AVAILABILITY OF NITROGEN BY THE ALKALINE PERMANGANATE METHOD.

By E. W. MAGRUDER (F. S. Royster Guano Co., Norfolk, Va.).

During the past year the writer had occasion to study a number of analyses of fertilizer materials and of mixed goods by the alkaline permanganate method and to check results with a number of other chemists. Your attention is directed to some of the problems presented.

At the meeting of the American Chemical Society in September, C. S. Robinson presented a paper on this subject, and his work shows that he is going to the very root of the subject. His results do not seem to uphold the official method[1] from a scientific standpoint, for where a method designed to show the availability of organic compounds gives urea an availability of not over 20 per cent, as is shown by his results, it would seem that something is fundamentally wrong.

Our official method says to digest with permanganate for not less than 30 minutes, gradually raise the temperature and, after danger of frothing has passed, distil. This leaves a very wide latitude for digestion. It was found with many samples that if 30 minutes only were allowed for digestion, so much frothing occurred that the time of distillation was greatly increased. In a strong alkaline permanganate solution, the time of digestion ought to make a considerable difference in the amount of nitrogen given off, so that two people following the official method would get varying results, depending upon the time taken in the digestion. It is suggested that the directions be made more specific.

TABLE 1.

*Determination of water-insoluble nitrogen.*

| Sample No. | CATHCART* | ROYSTER GUANO CO. | HASKINS† | CARPENTER‡ | JONES¶ |
|---|---|---|---|---|---|
| | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* |
| 1 | 0.49 | 0.64 | .... | .... | .... |
| 2 | 0.22 | 0.37 | .... | .... | .... |
| 3 | 0.57 | 0.67 | .... | .... | .... |
| 4 | 1.06 | 1.22 | .... | .... | .... |
| 5 | .... | 1.04 | 0.94 | .... | .... |
| 6 | 0.27 | 0.35 | .... | 0.23 | 0.41 |
| 7 | 0.30 | 0.45 | .... | 0.34 | 0.49 |

*C. S. Cathcart, New Brunswick, N. J.
†H. D. Haskins, Amherst, Mass.
‡F. B. Carpenter, Richmond, Va.
¶C. H. Jones, Burlington, Vt.

As permanganate acts on organic matter which does not contain nitrogen, much of it is used up on other than nitrogenous materials, and as large samples of low-grade materials have to be used, the large

[1] *Assoc. Official Agr. Chemists, Methods*, 1920, 11.

amount of organic matter present may account for the low availability.

A determination of the water-insoluble nitrogen should result in concordant results, but the experience of the writer has been that chemists get as great differences on water-insoluble nitrogen as on the permanganate available, as the following results show:

Some of these differences may not be considered great, but when the small amount of nitrogen is taken into consideration, the percentage of activity is changed materially. If the chemists can not agree on the water insoluble, no matter how closely they agree on the permanganate active, there will be a wide difference in the results on permanganate inactive and the percentage of activity.

The variation of results due to the use of different makes of filter paper is a point worth considering.

TABLE 2.

*Differences in percentage of activity caused by small differences in water insoluble and permanganate active.*

| ANALYST | WATER INSOLUBLE | PERMANGANATE | | AVAILABILITY |
|---|---|---|---|---|
| | | Active | Inactive | |
| | *per cent* | *per cent* | *per cent* | *per cent* |
| Cathcart | 0.22 | 0.12 | 0.10 | 53.60 |
| Royster Guano Co. | 0.31 | 0.14 | 0.17 | 45.10 |
| Haskins | 0.62 | 0.27 | 0.35 | 43.55 |
| Royster Guano Co. | 0.59 | 0.31 | 0.28 | 52.54 |
| Carpenter | 0.23 | 0.15 | 0.08 | 64.30 |
| Cathcart | 0.27 | 0.13 | 0.14 | 48.10 |
| Jones | 0.41 | 0.17 | 0.24 | 42.00 |
| Royster Guano Co. | 0.35 | 0.16 | 0.19 | 45.20 |
| Carpenter | 0.34 | 0.16 | 0.18 | 47.10 |
| Cathcart | 0.30 | 0.19 | 0.11 | 63.30 |
| Jones | 0.49 | 0.22 | 0.27 | 45.00 |
| Royster Guano Co. | 0.45 | 0.22 | 0.23 | 47.30 |

The differences shown are well within experimental error, yet the percentages of activity vary so widely that one analyst would condemn and the other pass the same goods and both with wide margins.

The interpretation of results also needs study and modification. At present the activity of the nitrogen in fertilizer materials is based on the relation between the water insoluble and permanganate active; it leaves entirely out of consideration the water-soluble nitrogen, the most valuable portion. It would be better to calculate the value of the nitrogen by comparing the permanganate active plus the water soluble with the total.

TABLE 3.

*Availability of nitrogen by the alkaline permanganate method.*

| SAMPLE | TOTAL NITROGEN | WATER SOLUBLE | WATER INSOLUBLE | PERMANGANATE AVAILABLE | PERMANGANATE INACTIVE | ACTIVITY | ACTIVITY BASED ON TOTAL NITROGEN |
|---|---|---|---|---|---|---|---|
| | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* |
| 1 | 9.16 | 1.26 | 7.90 | 5.82 | 2.08 | 73.67 | 77.29 |
| 2 | 8.83 | 6.36 | 2.47 | 1.24 | 1.23 | 50.20 | 86.36 |

It should be noted that Sample No. 2 has the higher water-soluble and lower permanganate inactive nitrogen, which should indicate the better source of nitrogen, but by the present method of interpretation it has much the lower percentage of activity, which indicates a poorer source of nitrogen.

## REPORT ON POTASH[1].

By J. T. FOY (Clemson Agricultural College, Clemson College, S. C.), *Associate Referee.*

It is recommended—

(1) That the method by Moore and Caldwell[2] which calls for the use of stronger alcohol in connection with the Lindo-Gladding method be studied further. This was recommended at the last meeting but no samples were sent out to collaborators.

(2) That as the centrifugal method for determining potash, by Elmer Sherrill[3], seems to be applicable when a rapid determination for factory control is necessary, it is worthy of consideration, and should be given a trial by the association. However, this method can not compare with the Lindo-Gladding method for official use.

## THE DETERMINATION OF SMALL AMOUNTS OF POTASH BY THE LINDO-GLADDING METHOD.

By WILLIAM HAZEN (Bureau of Soils, Washington, D. C.).

A few years ago an investigation was made by Ross, Merz and Wagner of the Bureau of Soils[4] on the recovery of potash as a by-product in the cement industry. Recently a corresponding survey was made of the blast-furnace industry. In these investigations numerous samples of various materials were analyzed for potash. All analyses were made

[1] Presented by R. N. Brackett.
[2] *J. Ind. Eng. Chem.*, 1920 **12**: 1188.
[3] *Ibid.*, 1921, **13**: 227.
[4] U. S. Dept. Agr. Bull. **572**: 1917.

in the usual way by fusion in J. L. Smith crucibles and subsequent treatment by the official Lindo-Gladding method[1].

The potash content of most of the samples analyzed was found to be quite low, often less than 0.1 per cent. It was felt, therefore, that special accuracy was required in this work as any constant error in the results, although actually small, might have a relatively large effect on the final estimates. In order to secure the greatest accuracy all samples were analyzed by two chemists. When their results differed by as much as 0.1 per cent the sample was analyzed by a third chemist.

The claim made by some authorities that the use of 80 per cent alcohol—as called for in the official method—gave low results was, of course, well known, but at the time these investigations were undertaken the consensus of opinion seemed to be that the error arising from this source was negligible.

Recently attention was again directed to this question. Moore and Caldwell[2] found that when used in determining potash *in the presence of sodium salts* 80 per cent alcohol gave low results, but that alcohol of this strength was entirely satisfactory in the absence of sodium salts. To explain this discrepancy, the view was advanced that when sodium salts are present they form a solution with the alcohol, and that it is the alcoholic sodium solution, rather than the alcohol alone, which exerts a solvent action on the chloroplatinate precipitate and causes low results in the determinations. As this sodium solution is not formed so readily in 95 per cent alcohol, Moore and Caldwell found that by using 95 per cent alcohol in the initial washing, this solubility error may be avoided. After the sodium salts have been removed by means of the Lindo-Gladding (ammonium chloride) solution, 80 per cent alcohol may be used without danger of dissolving any of the precipitate.

As soda is associated with potash in all the raw materials used in the cement and blast-furnace industries it was felt that the claims of Moore and Caldwell required further attention, owing to the bearing they might have on the investigations on the recovery of potash in these industries.

Accordingly, standard chloride and sulfate solutions of potassium and sodium were prepared from C. P. chemicals which had been further purified by recrystallization. Mixtures of these solutions were then taken in which the potash varied from about 1 to 50 milligrams. Two sets of determinations were made, in one of which the potash was associated with varying amounts of sodium sulfate and in the other with sodium chloride. Each set was divided into two groups, the excess platinic chloride being removed with 80 per cent alcohol in one group

[1] *Assoc. Official Agr. Chemists, Methods*, 1920, 12.
[2] *J. Ind. Eng. Chem.*, 1920, 12: 1188.

and with 95 per cent in the other. The final washing, after the ammonium chloride treatment, was made throughout with 80 per cent alcohol. Both groups of determinations were run at the same time so as to restrict the varying factors to the two mentioned, viz., the effect of the sodium salts and the strength of the alcohol used in washing the precipitate.

TABLE 1.

*Determination of potash using different concentrations of alcohol in the initial washing.*

| AMOUNTS TAKEN | | POTASSIUM OXIDE FOUND | |
|---|---|---|---|
| Potassium Oxide (as Sulfate) | Sodium Oxide (as Sulfate) | 80% Alcohol—Ammonium Chloride—80% Alcohol | 95% Alcohol—Ammonium Chloride—80% Alcohol |
| *gram* | *gram* | *gram* | *gram* |
| 0.0025 | ..... | 0.00235 | 0.00233 |
| 0.0025 | 0.0170 | 0.00217 | 0.00237 |
| 0.0025 | 0.0170 | 0.00217 | 0.00231 |
| 0.0050 | ..... | 0.00463 | 0.00482 |
| 0.0050 | 0.0060 | 0.00465 | 0.00491 |
| 0.0500 | ..... | 0.04990 | 0.05050 |
| 0.0500 | 0.1760 | 0.04910 | 0.04950 |
| 0.0500 | 0.3500 | 0.04890 | 0.04960 |
| potassium oxide (as chloride) | sodium oxide (as chloride) | | |
| 0.00500 | ..... | 0.00474 | 0.00485 |
| 0.00500 | 0.0160 | 0.00456 | 0.00493 |
| 0.00125 | ..... | 0.00105 | 0.00110 |
| 0.00125 | 0.0033 | 0.00098 | ..... |
| 0.00125 | 0.0055 | 0.00090 | 0.00121 |
| 0.03000 | 0.0200 | 0.02940 | 0.02980 |
| 0.04000 | 0.0500 | 0.03900 | 0.04010 |

The results given in Table 1, which represent in each case the average of two to six closely agreeing determinations, show that for the quantities of potash used low results were obtained in all cases when the first washing was made with 80 per cent alcohol. The actual error in these determinations is not great, but the percentage error when working with small amounts is quite appreciable. However, the sodium salts, both sulfates and chlorides, even when present in relatively large amounts, affected the determinations but slightly. In many cases the results were just as low with the potash salts alone as when appreciable amounts of sodium salts were added.

The values obtained in these determinations are, therefore, in agreement with the observations made by Moore and Caldwell regarding low results with 80 per cent alcohol but not with their explanation that this is mainly due to sodium salts.

When the first washing was made with 95 per cent alcohol the results were somewhat better, but even in this case they were slightly but consistently low, a fact which could only be explained on the assumption

that the alcohol (80 per cent), which was used subsequent to the ammonium chloride treatment, exerted a solvent action on the potash precipitate. To ascertain the correctness of this theory determinations were made in which 95 per cent alcohol was used both before and after the ammonium chloride treatment. The same samples were also determined by the two treatments shown in Table 1. The results of these determinations are given in Table 2.

TABLE 2.

*Determination of potash using different concentrations of alcohol in both the initial and final washings.*

| AMOUNTS TAKEN | | POTASSIUM OXIDE FOUND | | |
|---|---|---|---|---|
| Potassium Oxide (as Sulfate) | Sodium Oxide (as Sulfate) | 80% Alcohol—Ammonium Chloride—80% Alcohol | 95% Alcohol—Ammonium Chloride—80% Alcohol | 95% Alcohol—Ammonium Chloride—95% Alcohol |
| *gram* | *gram* | *gram* | *gram* | *gram* |
| 0.00125 | . . | 0.00105 | 0.00109 | 0.00127 |
| 0.00125 | 0.0020 | 0.00103 | 0.00110 | 0.00124 |
| 0.00125 | 0.0030 | 0.00104 | 0.00108 | 0.00128 |
| 0.00500 | . . | 0.00440 | 0.00482 | 0.00492 |

In Table 2 it is seen that the 95 per cent method gives results that agree almost exactly with the theoretical amounts taken, while the results obtained with the other two methods are appreciably lower, as was to be expected from the results given in Table 1. The effect of the sodium salts is again shown to be quite negligible, and the logical conclusion seems to be that the error observed in using 80 per cent alcohol is mainly due to the solubility of the potash precipitate in alcohol of that strength.

Some determinations made with 90 per cent alcohol showed that it may be safely used, and that it has the advantage over the 95 per cent solution in that fewer washings are required to free the precipitate from ammonium chloride and other foreign matter. Thus, using 0.0050 gram of potassium oxide (as $K_2SO_4$) and 0.0100 gram of sodium oxide (as

TABLE 3.

*Determination of potash in iron ores.*

| Sample No. | POTASSIUM OXIDE | |
|---|---|---|
| | 80% Alcohol—Ammonium Chloride—80% Alcohol | 90% Alcohol—Ammonium Chloride—90% Alcohol |
| | *per cent* | *per cent* |
| 533 | 0.120 | 0.128 |
| 539 | 0.147 | 0.205 |
| 541 | 0.200 | 0.248 |
| 542 | 0.140 | 0.170 |

$Na_2SO_4$), four determinations in which 90 per cent alcohol was used both before and after the ammonium chloride treatment gave results averaging 0.00505 gram of potassium oxide.

In Table 3 are given some comparative results obtained in the analysis of samples of iron ore. These again show that 80 per cent alcohol gives lower results than the 90 per cent solution. However, the differences in the two sets of determinations are not sufficiently great to have any serious effect on the potash recovery investigations to which reference has been made.

## SUMMARY.

In the determination of potash 90 per cent alcohol gives better results than the 80 per cent solution, and *when working with small amounts of potash* and *high accuracy is desired* it is advisable to use the stronger alcohol, *both before and after the ammonium chloride treatment.* However, it takes a longer time to wash out ammonium chloride salts with the 90 per cent solution. Furthermore, in ordinary fertilizer work the samples generally contain relatively high amounts of potash, in which case the percentage error arising from the use of 80 per cent alcohol will not be very serious. Therefore, in ordinary work the best procedure would be to use 90 per cent alcohol in the initial and 80 per cent in the final treatment.

No report on potash availability was made by the associate referee.

# REPORT ON PRECIPITATED PHOSPHATES.

By H. D. HASKINS (Agricultural Experiment Station, Amherst, Mass.), *Associate Referee.*

Following the recommendation of the association in 1920, that the method for the determination of available phosphoric acid in precipitated phosphate be studied further, the work was continued as outlined:

(1) Comparison of results obtained by the use of 1- and 2-gram charges, according to manipulation as outlined in the official methods for the determination of soluble and insoluble phosphoric acid in fertilizers[1].

(2) The effect of two successive treatments, each employing 100 cc. of neutral ammonium citrate, on a 2-gram charge of the precipitated phosphate. (The properly washed residue obtained after the first treatment of the precipitated phosphate with the citrate solution is introduced into the Erlenmeyer flask together with the filter paper and the whole treated with a second application of 100 cc. of neutral ammonium citrate, previously heated to 65° C. Manipulation to be the same as for the first treatment.)

[1] *Assoc. Official Agr. Chemists, Methods,* 1920, 4.

TABLE 1.

*Results obtained in a study of methods for the determination of phosphoric acid in precipitated phosphate.*

| ANALYST | WATER-SOLUBLE PHOSPHORIC ACID* | | CITRATE-INSOLUBLE PHOSPHORIC ACID* | | | TOTAL PHOSPHORIC ACID* |
|---|---|---|---|---|---|---|
| | 2-Gram Charge | 1-Gram Charge | 2-Gram Charge | 1-Gram Charge | 2-Gram Charge (2 extractions) | |
| *S. & S. No. 597* | | | | | | |
| | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* |
| Percy O'Meara, Agricultural Experiment Station, E. Lansing, Mich. | 1.31 | 1.81 | 10.93 | 4.94 | 1.32 | 41.27 |
| L. S. Walker, Amherst, Mass. | 1.68 | 2.00 | 12.21 | 6.82 | 1.99 | 41.15 |
| *Whatman No. 1.* | | | | | | |
| F. B. Carpenter, Virginia-Carolina Chemical Co., Richmond, Va. | 0.95 | 1.23 | 12.76 | 8.45 | 3.88 | 40.90 |
| William Hazen, Bureau of Soils, Washington, D. C. | 1.97 | . | 4.50 | .... | .... | 41.26 |
| L. S. Walker | 1.63 | 2.09 | 12.17 | 6.69 | .... | .... |
| J. H. Parkins, Royster Guano Co., Norfolk, Va. | 1.49 | 1.93 | 10.51 | 3.83 | 0.07† | 41.42 |
| *Whatman No. 2.* | | | | | | |
| Percy O'Meara | 1.36 | 1.86 | 9.40 | 4.38 | 1.10 | |
| L. S. Walker | 1.58 | 2.11 | 11.60 | 6.99 | 2.28 | |
| *Munktell's Swedish No. 1-F.* | | | | | | |
| F. B. Carpenter | 0.94 | 1.30 | 12.87 | 8.37 | 3.43 | |
| William Hazen | 1.90 | .... | 5.90 | .... | .... | |
| L. S. Walker | 1.65 | 2.17 | 12.11 | 6.89 | .... | |
| J. H. Parkins | 1.59 | 1.91 | 10.52 | 3.83 | 0.07† | |
| *Munktell's Swedish No. 2.* | | | | | | |
| Percy O'Meara | 1.38 | 1.84 | 9.47 | 4.58 | 1.19 | |
| L. S. Walker | 1.63 | 2.14 | 12.21 | 7.12 | 1.75 | |
| *Durieux French No. 121.* | | | | | | |
| F. B. Carpenter | 1.17 | 1.53 | 13.07 | 7.78 | 4.06 | |
| William Hazen | 2.02 | .... | 3.62 | .... | .... | |
| L. S. Walker | 1.75 | 2.35 | 11.90 | 6.92 | .... | |
| J. H. Parkins | 1.73 | 2.05 | 10.23 | 3.80 | 0.07† | |

*Average of 2 determinations.
† 1-gram charge used.
NOTE.—L. S. Walker, using the Wagner method (500 cc. of 2% citric acid to a charge) and S. & S. No. 597 filter paper, obtained the following results: 5-gram charge—30.23%; 2½-gram charge—40.65% of available phosphoric acid.

(3) To study the effect of a 2% citric acid solution on the precipitated phosphate. Manipulation to be according to the tentative Wagner method[1] for the determination of the citrate soluble phosphoric acid in basic slag.

(4) To study the adaptability of the various grades of paper for the filtration after the digestion of the 2-gram charge of the precipitated phosphate. (Experience has shown that the S. & S. No. 597 filter paper which was familiar to most chemists in prewar times was particularly adapted for this use. Many other papers have been found too porous, allowing the very fine particles of precipitated phosphate to pass through. The difficulty in securing S. & S. No. 597 filter paper of the same quality as formerly seems to warrant the accumulation of experimental data with as large a variety of papers as possible, with a view to selecting a satisfactory substitute).

Samples were prepared and sent to seven chemists. Five reported the detailed results shown in Table 1.

The wide difference in the results of four of the chemists is due largely to variation in neutrality and strength of the ammonium citrate used. Therefore the results of all analysis should be studied separately before drawing conclusions.

A comparison of the results for insoluble phosphoric acid, using 2- and 1-gram charges, manipulation according to the official method with neutral ammonium citrate as a solvent, is shown in Table 2.

TABLE 2.

*Comparison of results for insoluble phosphoric acid.*

| ANALYST | INSOLUBLE PHOSPHORIC ACID | | | 1-Gram Charge (Percentage Decrease in Insoluble Phosphoric Acid) |
|---|---|---|---|---|
| | 2-Gram Charge | 1-Gram Charge | 2-Gram Charge (2 extractions) | |
| | *per cent* | *per cent* | *per cent* | |
| L. S. Walker | 12.03* | 6.91* | 2.01* | 42.5 |
| Percy O'Meara | 9.93 | 4.63 | 1.20 | 53.0 |
| F. B. Carpenter | 12.90 | 8.20 | 3.79 | 36.0 |
| William Hazen | 4.67 | .... | .... | .... |
| J. H. Parkins | 10.42 | 3.82 | 0.07† | 63.3 |

* Average of 12 tests; all other figures show averages of 6 tests.
† 1-gram charge used.

The results given in Table 2 show that a much lower percentage of insoluble phosphoric acid is obtained by using a 1-gram than a 2-gram charge, manipulation according to the official method, the percentage decrease varying from 36.0 to 63.3. A 2-gram charge subjected to 2 successive extractions of 100 cc. each of neutral ammonium citrate gives even lower percentages of insoluble phosphoric acid, but the writer is of the opinion that the extra manipulation which the modified method entails would hardly warrant serious consideration of its adoption by

[1] *Assoc. Official Agr. Chemists, Methods*, 1920, 14.

the association. The results, however, emphasize the inadequacy of the present official method for the treatment of this class of materials.

NOTE.—Since this report was presented the writer, acting upon suggestions of Committee A, has had this phase of the work amplified. One gram of the phosphate subjected to 2 successive treatments of 100 cc. each of neutral ammonium citrate gave 0.06% of insoluble phosphoric acid as an average of 3 determinations. A similar test made in the laboratory of the Royster Guano Company gave 0.07% as an average of 6 tests (Table 2). The use of a ½-gram charge with 2 successive treatments of 100 cc. each of neutral citrate solution gave but a mere trace of insoluble phosphoric acid.

Results which form a part of Table 1 were secured at the Massachusetts Agricultural Experiment Station by the treatment of 5- and 2½-gram charges with 500 cc. of a 2 per cent solution of citric acid, according to the tentative Wagner method. The results obtained with a 5-gram charge on available phosphoric acid correspond with those secured by the official method employing a 2-gram charge with neutral ammonium citrate. The Wagner method with a 2½-gram charge shows practically all of the phosphoric acid in available form. It is considered that this method (introduced for the treatment of basic phosphate products which have a tendency to decompose the neutral ammonium citrate solution) is not applicable for the treatment of precipitated phosphate which is neutral or slightly acid in reaction.

The results of a study by Walker of Anaconda treble superphosphate as to the effect on the available phosphoric acid by using a 1-gram charge, with manipulation according to the official method, are shown as follows, expressed in percentage:

| | |
|---|---|
| Moisture | 3.11 |
| Total phosphoric acid | 47.82 |
| Water-soluble phosphoric acid | |
| 1 gram washed with 250 cc. of water | 41.59 |
| 2 grams washed with 250 cc. of water | 41.46 |
| Insoluble phosphoric acid | |
| 1 gram treated with 100 cc. of neutral citrate | 2.22 |
| 2 grams treated with 100 cc. of neutral citrate | 2.26 |

Attention is called to the fact that with materials of this class, the employment of a 1-gram charge apparently does not give any lower insoluble phosphoric acid than does the use of 2 grams. This would bear out the theory that the high insoluble phosphoric acid tests on precipitated phosphate which follow the use of a 2-gram charge with neutral ammonium citrate are due largely to the over-saturation of the citrate solution with phosphate of lime.

Results secured by the various collaborators would indicate that any one of the six filter papers tested was fairly well adapted to the work,

although it was observed that the Durieux No. 121 was considerably slower than the others, and if many tests were to be made it might be advisable to use one of the Whatman papers, preferably No. 2, or the S. & S. No. 597.

## SUMMARY.

The conclusions to be drawn from these studies coincide with the conclusions of the preceding year. Both emphasize the inadequacy of the present official method for the analysis of the precipitated phosphates.

It may be pointed out—

(1) That satisfactory results are obtained in the determination of available phosphoric acid by the present official method on fertilizer mixtures in which precipitated phosphate has been used as a source of available phosphoric acid.

(2) That precipitated phosphate receives the preference of the average Connecticut Valley tobacco grower, both as a source of phosphoric acid in fertilizer mixtures and where the purchase of chemicals and crude stock materials is the custom.

(3) That results of vegetation tests described in the report on precipitated phosphate for 1920 show this product to be a good source of available phosphoric acid.

Your associate referee is of the opinion that further work on the subject is not warranted.

## RECOMMENDATIONS.

It is recommended—

(1) That the determination of insoluble phosphoric acid in precipitated phosphates be carried out according to the present official method for the determination of insoluble phosphoric acid in fertilizers[1], with the exception that a 1-gram charge be employed.

(2) That a perforated platinum crucible and gentle suction be employed in the filtration of the citrate solution after treatment, and that a filter paper be employed that will insure a free and rapid filtration without allowing the finely divided particles to pass through. The following papers have been found satisfactory (and there may be others): S. & S. No. 597, Whatman No. 2, Whatman No. 1, Munktell's No. 1-F, Munktell's No. 2 and Durieux No. 121.

W. H. Ross, C. B. Durgin and R. M. Jones (Bureau of Soils, Washington, D. C.), presented a paper on "The Composition of Commercial Phosphoric Acid"[2].

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[1] *Assoc. Official Agr. Chemists, Methods*, 1920, 4.
[2] *J. Ind. Eng. Chem.*, 1922, 14: 533.

# THE DETERMINATION OF EXTREMELY SMALL AMOUNTS OF PHOSPHORUS BY THE OFFICIAL METHOD[1].

By R. C. WILEY (University of Maryland, College Park, Md.).

In the course of research work last winter, the writer had occasion to determine phosphorus with extreme accuracy. The following application of the official volumetric method[2] was formulated. No originality is claimed, but the scheme is given with the hope that it will prove useful to others who are doing similar work.

Bowser[3], Raper[4], Serger[5] and Veitch[6] are prominent among the chemists who have worked upon the determination of small amounts of phosphorus. All used the molybdate method or some modification of it.

The method used by the writer is as follows:

Measure an aliquot of the solution into a beaker and add 20 cc. of a saturated solution of ammonium nitrate and enough water to make the total volume about 75 cc. Heat the solution to 55°C. in a water bath and add ammonium molybdate solution of the same temperature. After 15 minutes filter off the precipitate and carefully wash free from acid with distilled water. (It has been found that it is quite possible to have the washings free from acid and yet have acid on the top of the filter paper, especially under the folds, and it is always well to test these localities carefully before pronouncing the precipitate free from acid.) Carefully transfer the precipitate and paper into the beaker and dissolve in 0.2N sodium hydroxide. Add phenolphthalein. Neutralize the solution with 0.1N sulfuric acid and from the difference in readings calculate the amount of phosphorus.

One of the most essential points in the determination is to refrain from pulping or unduly tearing the filter paper in dissolving the precipitate or in subsequent titration with the standard acid. If this precaution is not taken the color of the indicator is occluded to such an extent by the filter paper fibers that the end point can not be distinctly seen, whereas if the filter paper fiber is not pulped the exact point of the change from alkalinity to acidity can be seen. That such brilliancy as the alkaline color of phenolphthalein should be masked by the occlusion of white filter paper fiber may seem strange, but such is the case.

As 1 cc. of 0.1N solution is equivalent to 0.00013 gram of phosphorus, and the error in reading is not more than 0.1 to 0.05 cc., it follows that a difference as little as 0.01 milligram of phosphorus can be detected.

The following results show how pulping the filter paper fiber causes variation in results:

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[1] Presented by R. B. Deemer.
[2] *Assoc. Official Agr. Chemists, Methods*, 1920, 3.
[3] *Am. Chem. J.*, 1911, **45**: 230.
[4] *Biochem. J.*, 1914, **8**: 320.
[5] *Chem. Ztg.*, 1915, **39**: 613.
[6] U. S. Bur. Chem. Bull. **90**: (1905), 188

*Phosphorus.*

| SOLUTION | PAPER PULPED | VARIATION | PAPER NOT PULPED | VARIATION |
|---|---|---|---|---|
| | *mgs.* | *mg.* | *mgs.* | *mg.* |
| | 19.42 | | 19.24 | 0.02 |
| | 19.39 | | 19.26 | |
| 1 | 19.45 | | | |
| | 19.20 | 0.25 | | |
| | 6.46 | | 6.32 | 0.00 |
| 2 | 6.21 | | 6.32 | |
| | 6.31 | | | |
| | 6.42 | 0.25 | | |
| | 3.17 | | 3.27 | |
| 3 | 3.25 | | 3.28 | 0.01 |
| | 3.24 | | | |
| | 3.38 | 0.21 | | |
| | 3.31 | | 3.26 | 0.01 |
| 4 | 3.25 | | 3.27 | |
| | 3.44 | | | |
| | 3.35 | 0.19 | | |

The tabluated results show clearly the disadvantage of pulping the filter paper in the determination.

The following results, expressed as milligrams, were obtained in determining the amount of phosphorus in the washings from silica gel:

| | SAMPLE NO. 1. | SAMPLE NO. 2. |
|---|---|---|
| 1st washing | 4.15 | 4.74 |
| 11th washing | 0.10 | 0.14 |
| 21st washing | 0.06 | 0.12 |
| 31st washing | 0.06 | 0.12 |
| 41st washing | 0.05 | 0.10 |

The foregoing results, the writer believes, show that the official method can be used in this way for the rapid determination of very small amounts of phosphorus.

# REPORT ON INORGANIC PLANT CONSTITUENTS

## (Calcium, magnesium, iron and aluminium in the ash of seed).

By A. J. PATTEN (Michigan Experiment Station, E. Lansing, Mich.), *Referee.*

The work carried on during the past year followed the recommendations made by the association at its last meeting.

For the determination of calcium, magnesium and manganese in the ash of seeds the methods[1] that have been before the association since 1916 gave satisfactory results. A method for the determination of iron and aluminium in the filtrate from the magnesium determination was also studied in the referee's laboratory. For this work a hydrochloric acid solution representing the approximate composition of the ash of seeds was prepared with the following ingredients, expressed as grams per 1000 cc.:

| | |
|---|---|
| Phosphorus pentoxide ($P_2O_5$) | 4.500 |
| Calcium oxide (CaO) | 0.500 |
| Magnesium oxide (MgO) | 0.800 |
| Manganomanganic oxide ($Mn_3O_4$) | 0.020 |
| Aluminum oxide ($Al_2O_3$) | 0.112 |
| Ferric oxide ($Fe_2O_3$) | 0.168 |
| Potassium oxide ($K_2O$) | 2.500 |
| Sodium oxide ($Na_2O$) | 1.400 |
| Total | 10.000 |

Results of collaborative tests are shown in the following table:

*Results on synthetic solution.*

| ANALYST | CALCIUM OXIDE | MAGNESIUM OXIDE | MANGANO-MANGANIC OXIDE |
|---|---|---|---|
| | *per cent* | *per cent* | *per cent* |
| I. H. Hopper, Agricultural College, N. Dakota | 4.85 | 8.40 | 0.22 |
| O. B. Winter, Agricultural Experiment Station, E. Lansing, Mich. | 4.96 | 8.22 | 0.21 |
| M. L. Grettenberger, Agricultural Experiment Station, E. Lansing, Mich. | 5.02 | 8.56 | 0.20 |
| A. J. Patten | 4.86 | 8.22 | 0.22 |
| Average | 4.92 | 8.35 | 0.21 |
| Theory | 5.00 | 8.00 | 0.20 |
| Difference | 0.08 | 0.35 | 0.01 |

The results for calcium and manganese are very good indeed, but those for magnesium are invariably high due, probably, to occlusion of small amounts of iron or aluminium phosphate.

[1] *J. Assoc. Official Agr. Chemists*, 1921, 4: 392.

### IRON AND ALUMINIUM.

For the determination of iron and aluminium in the ash of seeds it is necessary to precipitate them as phosphates, since it is altogether impracticable to remove the large amount of phosphoric acid. It is also necessary to remove the sodium citrate before the iron and aluminium can be precipitated. Evaporation with nitric acid alone or nitric and sulfuric acid did not prove entirely satisfactory in removing the sodium citrate, so the following procedure was tried:

The filtrate from the magnesium determination was evaporated to dryness after the addition of 15 cc. of nitric acid. Sulfuric acid and 1 cc. of perchloric acid were added and the solution again evaporated to dryness. The residue was taken up with hot water and hydrochloric acid, boiled, filtered, and the iron and aluminium precipitated by the addition of ammonium hydroxide and ammonium acetate.

The results obtained are encouraging and warrant further work.

### RECOMMENDATIONS.

It is recommended—

(1) That further work be done on the determination of calcium and magnesium in the ash of seeds.

(2) That the method for manganese as given in the body of this report be adopted as official.

(3) That further study be given to the determination of iron and aluminium in the ash of seeds.

## REPORT ON SULFUR AND PHOSPHORUS IN THE SEEDS OF PLANTS.

By W. L. LATSHAW (Agricultural Experiment Station, Manhattan, Kans.), *Associate Referee.*

During the past year work was attempted in accordance with the suggestions and recommendations adopted at the meeting of the association in 1920[1]. As a definite method had not been outlined, it was considered inadvisable to submit samples to other collaborators.

Three samples of seed products were used: Soy bean meal, cottonseed meal and mustard seed meal. As the mustard seed meal was extremely oily it was mixed with 50 per cent of potato starch.

Various amounts of the sample, from 0.2 of a gram to 1.0 gram, were tried; also from 0.2 to 0.8 gram of potassium chlorate, and from 10 to 18 grams of sodium peroxide. The larger amounts proving the more reliable, they were incorporated into the following procedure:

[1] *J. Assoc. Official Agr. Chemists*, 1921, 5: 136.

## ORGANIC AND INORGANIC SULFUR AND PHOSPHORUS IN THE SEEDS OF PLANTS

### APPARATUS.

Parr peroxide bomb.—For sulfur determination to be made independent of colorific process.

### REAGENTS.

(a) *Sodium peroxide* (free from sulfur and phosphorus).

(b) *Potassium chlorate*, finely ground.

### OXIDATION AND SOLUTION.

Weigh into the bomb a 1-gram sample of the seed under examination, ground to pass a ½-mm. sieve. Add in the order mentioned 0.7 gram of potassium chlorate and 15–17 grams of sodium peroxide. Seal the bomb and shake the charge thoroughly. Bring the base of the bomb into contact with a small but very hot flame from the blast lamp. (A sudden intensifying of the glow on the wall of the bomb indicates that the charge has been exploded.) After allowing the bomb to cool, transfer the contents to a beaker with the aid of a funnel and a stream of hot water; acidify 2% with hydrochloric acid. Filter off any unoxidized particles of carbon, and the filtrate is ready for the determination of sulfur.

### SULFUR.

Proceed as directed for sulfuric acid[1].

### PHOSPHORUS.

Evaporate the filtrate from the sulfur determination to a uniform volume, taking aliquot portions when the phosphorus expected is high, and proceed as directed by the official method[2].

The results on the various samples of cottonseed meal, soy bean meal and mustard seed meal by the foregoing procedure are as follows, expressed in percentage:

| | SULFUR | PHOSPHORUS |
|---|---|---|
| Cottonseed meal | 0.53 | 2.02 |
| | 0.52 | 1.98 |
| | 0.56 | 2.12 |
| | 0.49 | 2.14 |
| | 0.49 | 2.20 |
| | 0.52 | 2.14 |
| | 0.51 | 2.17 |
| Soy bean meal | 0.42 | 1.26 |
| | 0.43 | 1.28 |
| | 0.43 | 1.22 |
| | 0.39 | 1.22 |
| | 0.38 | 1.21 |
| | 0.41 | 1.31 |
| Mustard seed meal | 1.17 | 1.54 |
| | 1.16 | 1.60 |
| | 1.14 | 1.57 |
| | 1.08 | 1.59 |
| | 1.10 | 1.59 |
| | 1.06 | 1.60 |
| | 1.10 | 1.58 |

[1] *Assoc. Official Agr. Chemists, Methods*, 1920, 18.
[2] *Ibid.*, 3.

A small amount of carbon remained after the charge was neutralized and made acid. The carbon residues from 18 determinations averaged 0.51 per cent of the sample taken. This small amount was considered negligible so far as the phosphorus and sulfur were concerned.

Acting upon the suggestion of the referee, A. J. Patten, another method[1] was tried. Twelve determinations on the several meals were started at the same time, using the utmost care. Shortly after the heat was applied to the crucibles one of them exploded with violence, and before the analyst could turn the gas out ten more had exploded, all with considerable violence. The writer had used this method previously with some success in making determinations for sulfur in plant material and had hoped to get some figures for comparison. A lack of time prevented the making of additional trials.

It is recommended that the method as outlined be studied by the incoming referee and various other collaborators, in order that the results of their findings may determine the advisability of its adoption as an official procedure.

No general report on dairy products was made by the referee.

## THE CRYOSCOPIC EXAMINATION OF MILK.

By JULIUS HORTVET (State Dairy and Food Commission, St. Paul, Minn.), *Referee.*

The work of the referee during the past year was a continuation of the investigation conducted during the year 1920 and was in compliance with the recommendation adopted at the meeting held in November last. The plan of the work covered by the present report includes some features which have not heretofore been given special consideration—notably, the standardization of thermometers and the investigation of milk samples obtained from individual cows and herds known to be under pathologic disturbance, under unusual physical strain, or under abnormal conditions as to housing or feeding. Also it was deemed advisable to continue the systematic investigation of a number of series of samples mixed with known proportions of water in order to exhibit as fully as possible the true value of the cryoscopic method when applied alone or in conjunction with other methods which have been adopted as official or for some time have been regarded as standard. There was also borne in mind the necessity of giving due consideration to experimental errors, correction factors and tolerances justified under practical conditions. The general plan of the work outlined early in the present year is embodied in the following instructions issued to the collaborators:

[1] *J. Assoc. Official Agr. Chemists*, 1915, 1: 56.

## OUTLINE OF COLLABORATIVE WORK.

I. *Standardization of thermometer:*

(a) Location of freezing-point of pure water (true 0 of scale).

(b) Location of freezing-point of solution of 10 grams pure sucrose in pure water made up to 100 cc. at 20°C.

(c) Location of freezing-point of solution of 7 grams pure sucrose in pure water made up to 100 cc. at 20°C.

II. *Known-pure milk,* including 3 samples from individual cows and 3 samples from herds:

(a) Mix each milk with water in exact proportions, as follows: 5, 7, 9, 11, 13, 15 per cent by volume.

(b) Make determinations of specific gravity (at 60°F.), fat and solids-not-fat (calculated) on each milk sample and mixtures prepared therefrom.

(c) Determine lactose on each whole milk sample.

(d) Make freezing-point determinations[1] on each milk sample and on each mixture prepared therefrom.

(e) Make immersion refractometer readings (at 20°C.) on acetic serum and copper serum prepared from each milk sample and on each mixture prepared therefrom.

(f) Make ash determination on each acetic serum prepared in (e).

III. *Known-genuine milk,* including:

(a) Milk from individual cows of different breeds;

(b) Mixed milk of herds.

Make determinations of *specific gravity, fat, solids-not-fat* and *freezing-point*[1] on all samples.

[1]NOTE.—Make freezing-point determinations only on samples which are fairly sweet or fresh, i. e., samples which show an acidity test of not more than 0.01% or 0.02% above 0.15% (expressed in terms of lactic acid). Make the acidity determination according to the following method:

Measure out 17.6 cc. of sample using the 17.6 cc. Babcock pipet; dilute with an equal volume of water (free from carbon dioxide), washing out the pipet with the same; add 0.5 cc. of phenolphthalein indicator, and titrate with 0.1N sodium hydroxide. The number of cc. of 0.1N sodium hydroxide required to neutralize the sample of milk divided by 20 gives the percentage of lactic acid.

As a preparation for collaborative work on the cryoscopic examination of milk, it will be necessary to carry out a series of tests on the special thermometer to be used in the freezing-point determinations by means of the following: (1) A sample of recently boiled distilled water; (2) a solution consisting of 10 grams of pure sucrose dissolved in pure water made up to a volume of exactly 100 cc. at 20°C.; and (3) a solution consisting of 7 grams of pure sucrose dissolved in pure water made up to a volume of exactly 100 cc. at 20°C.

Each of the above determinations is to be repeated not less than 3 times.

As a preparation for the above determinations it is necessary that the collaborators devote a sufficient amount of time to preliminary trials for the purpose of becoming thoroughly familiar with the construction of the cryoscope and its method of operation. A description of the standard method of procedure is enclosed herewith.

A sample of pure sucrose can be obtained by application to the Director of the Bureau of Standards, Department of Commerce and Labor, Washington, D. C., The sucrose samples should be ordered promptly in order that there may be ample time for carrying out the outlined determinations.

There is also enclosed herewith a blank form on which the results of the freezing-point determinations are to be tabulated. Collaborators are required to report results to the referee, if possible, before February 15th. As soon as results have been obtained

**and the thermometer corrections worked out in comparison with a Bureau of Standards tested instrument, the general outline for collaborative work on samples of milk will be sent out. It is hoped that this first step which relates entirely to the testing and standardizing of thermometers will be attended to as promptly and carefully as possible.**

| Determinations | Recently Boiled Distilled Water Freezing-point ± W | SOLUTION OF 10 GRAMS PURE SUCROSE IN PURE WATER TO 100 cc. AT 20°C. | | SOLUTION OF 7 GRAMS PURE SUCROSE IN PURE WATER TO 100 cc. AT 20°C. | |
|---|---|---|---|---|---|
| | | Position of Observed Freezing-point (-S) | Freezing Point Depression S-W (Algebraic) | Position of Observed Freezing-point (-S) | Freezing Point Depression S-W (Algebraic) |
| 1st | | | | | |
| 2nd | | | | | |
| 3rd | | | | | |
| | | | | | |
| Averages | | XXXXXXXX | | XXXXXXXX | |

——————————Collaborator.

Express the results on the sucrose solutions as degrees freezing-point *depression* below the average of the observed freezing point obtained on the sample of pure water (±W), which may be above (+) or below (−) the 0-mark on the scale. In other words, each freezing-point depression of the sucrose solution will be obtained by the *algebraic* subtraction of the *average* of the freezing-point readings of pure water from each observed freezing-point of the sucrose solution. No corrections are to be applied in obtaining the freezing-point depressions, excepting as above stated, owing to the fact that all determinations are to be carefully carried out under the same conditions.

Prepare the sucrose solutions by dissolving, accurately weighed out, 10 grams and 7 grams respectively of pure sucrose in pure water, and make the solution up to a volume of exactly 100 cc. at 20°C.

NOTE.—Do not include in your report any adventitious results, i. e., results which are in marked disagreement with other results obtained by carefully following instructions. If any results appear to be erratic, they should be investigated and the tests carefully repeated.

Herewith I am sending you outline of instructions for A. O. A. C. collaborative work on the cryoscopy of milk. You have already received detailed instructions regarding Section I.—*Standardization of Thermometer*. It is desired that results which you have obtained on your thermometer be reported to the referee at an early date. The freezing-point readings on your thermometer are to be compared with readings obtained on a U. S. Bureau of Standards tested thermometer as given in the tabulation herewith enclosed. By comparing results on these two thermometers you will readily determine

whether your thermometer is sufficiently correct or whether it will be necessary to multiply by a correction factor in order to obtain correct results.

The remainder of the collaborative work you will find outlined in Sections II and III. Collaborators are requested as far as possible to complete all of the work included in the outlines. But, whenever it appears to be impossible to handle all the work, a choice may be made between Sections II and III. It is especially desirable that all collaborators carry out the plan of work outlined in Section III and as far as possible to undertake the work outlined for the samples described in Section II.

Samples of known-pure milk suitable for the collaborative work are to be obtained under careful supervision, i. e., in such a manner that there can be *no question regarding their genuineness*. It is not desired to give any attention to samples which are obtained from cows or herds which are known to be poorly fed or kept under such conditions that are not likely to yield wholesome marketable milk. In obtaining samples to be used for the purposes outlined in Section II it is directed that milk representing various breeds of cows be included. For example, milk from individual cows may represent various breeds such as Holstein, Jersey, Guernsey, Ayrshire, etc. The samples should represent as many different breeds of cattle as can conveniently be found in your locality, with the important requirement, as indicated above, that in all cases samples are known to be authentic and from properly fed healthy animals.

The analytical determinations are to be made in accordance with methods described in Official and Tentative A. O. A. C. Methods of Analysis, Revised to November 1, 1919. The lactose determinations are to be made by the gravimetric method—official, given in **XXI, 11.** The refractometric examinations are to be made according to methods described in **XXI, 16-18,** and the ash determinations are to be made according to method described in **16 (b)**. The freezing-point determinations are to be made according to the method described in the Journal of Industrial and Engineering Chemistry, March, 1921, pages 198-208. Determine specific gravity by means of an accurately graduated lactometer or hydrometer, or better by means of a Westphal balance, at 60°F. Make fat determinations by means of the Babcock method as described in Methods of Analysis, **XXI, 13, 14, 15,** and express results to 0.1 per cent. Calculate solids-not-fat from results obtained by the specific gravity and fat determinations. Repeat all doubtful determinations and do not include any results which appear to be erratic or questionable. It is desired that all results be checked very carefully and any results which appear to be unusual or questionable are to be investigated and verified.

Tabulate results obtained in Section II according to the form of tabulation shown in the article, "The Cryoscopy of Milk", Journal of Industrial and Engineering Chemistry, March 1921, pages 192-208.

## MISCELLANEOUS INSTRUCTIONS.

I. *Thermometer:*

(a) Examine the thermometer very carefully, using a lens if necessary, in order to determine whether any defects exist in the glass or in the mercury thread. Dislodge any particle of mercury which may be adhering to the inner surface of the space at the top of the stem. Also dislodge any gas bubble which may be noticeable in the bulb or which may form a separation at any part of the mercury thread. When the thermometer is brought into proper condition for use:

(b) Make standardization tests according to directions outlined under Section I of general instructions for collaborative work.

(c) Keep the thermometer always in upright position. In removing from stopper or reinserting in position do not turn the thermometer to an inverted position and avoid a horizontal position as much as possible. When the thermometer has once

been properly adjusted and carefully tested out it should be handled at all times with great care.

(d) Test out the thermometer at frequent intervals, once a week or more often, in order to keep an accurate record of any changes which may occur. Determine the true 0-position and the depression produced by a standard sucrose solution often enough to be certain at all times regarding the reliability of results.

II. *The cryoscope:*

(a) For a description of the cryoscope and its method of construction consult the Journal of Industrial and Engineering Chemistry, March 1921, pages 198-208.

(b) The apparatus should be set up as carefully and perfectly as possible. All connections should be sufficiently tight to avoid escape of ether vapors. Care should be taken to avoid breakage of the Dewar flask. The perforated loop at the lower end of the metal inlet tube should be adjusted to a position about 3 cm. above the bottom of the flask. The rubber tube connecting the air-drying tube with the air-inlet tube should be extended so as to cover the metal tube as far as the top surface of the cork. When removing the upper section of the cryoscope simply withdraw the glass tube which is inserted in the cork stopper at the top of the air-drying device.

(c) The bulb of the control thermometer should extend to a position about two-thirds of the distance between the surface of the 400 cc. ether level and the bottom. When the thermometer has once been properly inserted in the cork it should remain in position unless for special reason it may be necessary to withdraw it.

(d) Prepare a glass ether level gage of suitable length for inserting to within a short distance above the bottom of the flask. Insert over the upper end of the gage tube a short section of rubber tubing for the purpose of preventing breakage of the vacuum flask when the tube is inserted into the ether. The lower end of the tube should be provided with file marks indicating various ether levels, viz., 200 cc., 300 cc., 400 cc., etc.

(e) Place a small plug of cotton in the funnel tube (preferably a narrow short-stemmed thistle tube) for the purpose of separating impurities which may be present in the ether when being poured into the cryoscope.

(f) Pour into the air-drying tube only sufficient concentrated sulfuric acid to just cover the perforations in the small bulb near the bottom of the tube. Do not allow the sulfuric acid to rise to a level near the perforations at the shoulder of the mantle.

(g) The stirrer and freezing starter should both move freely in the metal tubes provided for them in the rubber stopper which holds the standard thermometer.

(h) Adjust the flow of water through the pump and regulate the pressure valve in such a manner that air will be forced through the apparatus at a fairly rapid rate, avoiding splashing or excessive foaming of the sulfuric acid. When all adjustments are properly made and a free passage of air is maintained through the apparatus it is possible to lower the temperature of the ether bath from approximately +20°C. to 0°C. in from 5 to 8 minutes. When the cooling action appears to be retarded the sulfuric acid must be removed from the drying tube and a fresh supply poured in.

(i) The ether drain tube on the other side of the cryoscope should carry off the vapors into the sink. No marked odor of ether should be noticeable at the top portion of the drain tube. If ether vapors are not drawn out perfectly, increase somewhat the length of the glass outlet tube which dips into the top of the drain. When the cryoscope is not in use place a plug of cotton in the top of the drain tube in order to check a tendency to vaporize. Remove the plug when the apparatus is in use.

(j) The glass tube at the back portion of the cryoscope stand is intended for holding the standard thermometer when it is removed from the freezing test tube. Place a pad of cork or rubber at the bottom of the tube to serve as a rest for the thermometer bulb.

The procedure followed in making the freezing-point determinations[1] has not been subjected to any material changes.

Replies were received from directors or chiefs in charge of eight laboratories signifying their willingness to assist in the collaborative work. But owing to difficulties arising from insufficient laboratory help, inability to provide necessary equipment, or pressure of official duties, a number of laboratories were unable to report results under any of the headings included in the outline of instructions. The referee was exceedingly fortunate in securing the assistance of the Milk Products Department of Libby, McNeill & Libby, Chicago, Ill., and of the chemists employed at the company laboratory located at Morrison, Ill. Also of great value as a contribution to these investigations was the cooperation of the Associate Referee on the Cryoscopy of Milk and his assistants at the State Agricultural Experiment Station, New Haven, Conn. A considerable amount of work was also contributed by the chemists employed in the laboratory of the Minnesota State Dairy and Food Department, St. Paul, Minn. The individuals who rendered valuable assistance during the past year and those who have been engaged in the collaborative work are the following:

*Libby, McNeill & Libby:* G. A. Menge, H. L. Germann, R. T. Beardsley and R. H. Tucker.

*Connecticut Agricultural Experiment Station:* E. M. Bailey, R. E. Andrew and R. T. Merwin.

*Minnesota Dairy and Food Department:* Henry Hoffman, Jr., Otto Kueffner and C. S. Corl.

Of first importance in any cryoscopic work is the careful standardization or testing of the thermometer. Not only is it necessary that the standard freezing-test thermometer be given careful attention and proper handling, but it is also of obvious consequence that the thermometer whereby the temperature of the cooling bath is controlled be also tested in order to insure its approximate accuracy. No cryoscopic tests of any kind should be attempted on a thermometer whose scale has not previously been calibrated and the necessary correction factors determined. Collaborators were therefore directed, as a preparatory procedure, to subject their thermometers to standardization tests after the manner outlined in the foregoing instructions.

The results of the thermometer tests are included in Table 1.

The freezing-point depressions obtained by means of standard sucrose solutions were applied for the purpose of correcting thermometer readings in the manner illustrated in Fig. 1 and in the accompanying tables. The illustrations are taken from two thermometers which yielded

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*J. Assoc. Official Agr. Chemists*, 1921, 5: 174.

extreme variations from the normal, but they are nevertheless all the more serviceable for the present purpose.

TABLE 1.

*Results of thermometer tests.*

| THERMOMETER | WATER | 7-GRAMS SUCROSE TO 100 CC. | 10-GRAMS SUCROSE TO 100 CC. | INTERVAL | CORRECTION FACTOR |
|---|---|---|---|---|---|
| | °C. | °C. | °C. | °C. | |
| Standard..... | +0.079 | −0.422 | −0.621 | 0.199 | ..... |
| No. 635..... | +0.030 | −0.428 | −0.626 | 0.198 | x1.005 |
| No. 2..... | +0.056 | −0.425 | −0.621 | 0.196 | x1.015 |
| No. 24M..... | +0.000 | −0.422 | −0.624 | 0.202 | x0.985 |
| No. 18..... | +0.011 | −0.430 | −0.630 | 0.200 | x0.995 |
| Conn..... | +0.022 | −0.422 | −0.622 | 0.200 | x0.995 |
| U. M.. ..... | +0.042 | −0.427 | −0.624 | 0.197 | x1.010 |
| No. 30..... | +0.035 | −0.432 | −0.627 | 0.195 | x1.020 |

The collaborative results obtained on samples of milk taken from individual cows and herds and on a number of samples taken under pathologic conditions are given in the report of the associate referee. It was deemed expedient to divide the subject in this manner in order that the numerous tests made under known conditions and on authentic samples together with the conclusions drawn therefrom might be presented in a distinct report. The results obtained on various sets of samples systematically mixed with known percentages of water are given in full in Tables 2, 3, 4 and 5.

Included in Table 5 are results obtained on a number of recent samples (chiefly market milks) which will bear a careful study and comparison in connection with the general discussion.

*Laboratory Thermometer No. 2.*

| WATER | 7 GRAM SUCROSE TO 100 cc. | 10 GRAM SUCROSE TO 100 cc. |
|---|---|---|
| +0.056°C. | −0.425°C. | −0.621°C. |

Interval = 0.196

0.196 equiv. 0.199

Correction = ×1.015

*Laboratory Thermometer No. 24*

| WATER | 7 GRAMS SUCROSE TO 100 cc. | 10 GRAMS SUCROSE TO 100 cc. |
|---|---|---|
| 0.00°C. | −0.420°C. | −0.625°C. |

Interval = 0.205

0.205 equiv. 0.199

Correction = ×0.971

*Example:*

Laboratory Thermometer No. 24.

F. pt. Depression Sample Milk = 0.548

(0.548 − 0.420) 0.971 = 0.124

Corrected depression = 0.422 + 0.124 = 0.546°C.

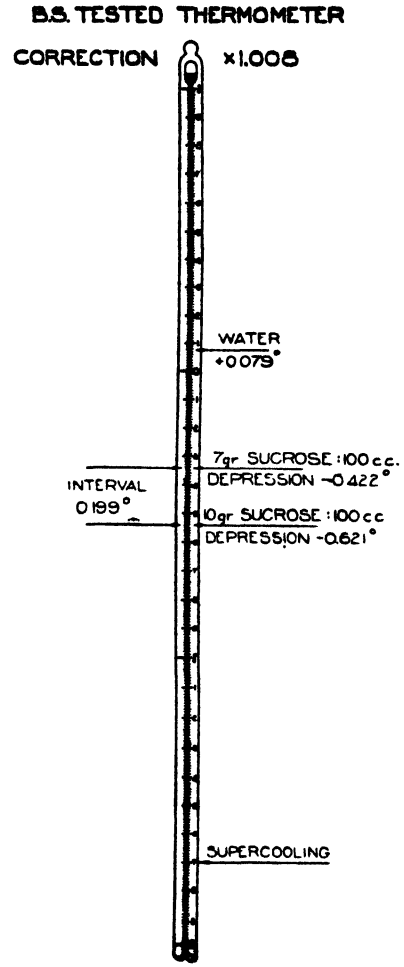


FIG. 1

NOTE.—Complete lactose determinations were made on all samples by only one of the collaborators in compliance with the instructions, but these results are not included in the tables, chiefly for the reason that they do not serve the purposes of the present stage of our work in the manner anticipated.

## DISCUSSION OF RESULTS.

Samples A, B, and C, Table 2, are Holstein milks and are much alike in general composition. Following the accepted rules of interpretation of results of the serum examination, it will be seen that the added water indications are similar in all three series of mixtures. The cryoscopic tests yield consistent and uniformly agreeing values in all cases except in the series based on Sample C, in which instance serious irregularities are apparent among the analytical results obtained on the mixtures containing, respectively, 11, 13, and 15 per cent of added water. Indications point to the possibility

TABLE 2.

*Milks containing known percentages of added water.*

(Connecticut Agricultural Experiment Station.)

| ADDED WATER | SPECIFIC GRAVITY 15.6°C. | FAT | SOLIDS NOT FAT | IMMERSION REFRACTOMETER READINGS 20°C. | | ASH IN ACETIC SERUM (Gram in 100cc.) | ADDED WATER INDICATED | CRYOSCOPIC EXAMINATION | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Acetic Serum | Copper Serum | | | Freezing point | Added Water $\frac{(T-T')100}{T}$ | Added Water $\frac{(0.550-T')100}{0.550}$ |
| **Sample A.—Individual cow—Holstein.** | | | | | | | | | | |
| *per cent* | | *per cent* | *per cent* | | | | | —0°C. | *per cent* | *per cent* |
| None | 1.0306 | 3.00 | 8.26 | 41.5 | 37.4 | 0.7880 | none | 0.545<br>0.545 | none | 0.91 |
| 5 | 1.0293 | 2.85 | 7.88 | 40.5 | 36.5 | 0.7520 | none | 0.516<br>0.516 | 5.32 | 6.18 |
| 7 | 1.0288 | 2.80 | 7.77 | 40.0 | 36.0 | 0.7320 | none | 0.504<br>0.504 | 7.52 | 8.36 |
| 9 | 1.0278 | 2.75 | 7.51 | 39.4 | 35.7 | 0.7164 | none | 0.492<br>0.492 | 9.72 | 10.54 |
| 11 | 1.0274 | 2.70 | 7.39 | 39.0 | 35.3 | 0.6980 | positive | 0.482<br>0.482 | 11.55 | 12.36 |
| 13 | 1.0268 | 2.65 | 7.24 | 38.5 | 34.9 | 0.6856 | positive | 0.470<br>0.471 | 13.66 | 14.45 |
| 15 | 1.0263 | 2.60 | 7.08 | 37.9 | 34.5 | 0.6736 | positive | 0.460<br>0.461 | 15.50 | 16.27 |
| **Sample B.—Herd, 11 Holsteins—April 13, 1921.** | | | | | | | | | | |
| None | 1.0313 | 3.70 | 8.56 | 41.8 | 37.5 | ..... | none | 0.535<br>0.535 | none | 2.72 |
| 5 | 1.0298 | 3.50 | 8.17 | 40.5 | 36.5 | ..... | none | 0.505<br>0.505 | 5.60 | 8.18 |
| 7 | 1.0286 | 3.40 | 7.84 | 39.8 | 36.1 | ..... | none | 0.494<br>0.495 | 7.57 | 10.09 |
| 9 | 1.0281 | 3.35 | 7.70 | 39.3 | 35.7 | ..... | Suspected | 0.484<br>0.484 | 9.53 | 12.00 |
| 11 | 1.0276 | 3.30 | 7.56 | 38.9 | 35.3 | ..... | positive | 0.472<br>0.473 | 11.68 | 14.09 |
| 13 | 1.0271 | 3.20 | 7.41 | 38.3 | 34.9 | ..... | positive | 0.462<br>0.462 | 13.64 | 16.00 |
| 15 | 1.0266 | 3.10 | 7.27 | 27.7 | 34.5 | ..... | positive | 0.452<br>0.453 | 15.42 | 17.73 |

TABLE 2.—Continued.

*Milks containing known percentages of added water.*

(Connecticut Agricultural Experiment Station.)

| ADDED WATER | SPECIFIC GRAVITY 15.6°C. | FAT | SOLIDS NOT FAT | IMMERSION REFRACTOMETER READINGS 20°C. | | ASH IN ACETIC SERUM (Gram in 100 cc.) | ADDED WATER INDICATED | CRYOSCOPIC EXAMINATION | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Acetic Serum | Copper Serum | | | Freezing point | Added Water $\frac{(T-T')\,100}{T}$ | Added Water $\frac{(0.550-T')\,100}{0.550}$ |
| **Sample C.—Herd, 11 Holsteins—May 2, 1921.** | | | | | | | | | | |
| *per cent* | | *per cent* | *per cent* | | | | | −0°C. | *per cent* | *per cent* |
| None | 1.0305 | 3.70 | 8.38 | 40.5 | 37.6 | 0.7396 | none | 0.540<br>0.540 | none | 1.82 |
| 5 | 1.0287 | 3.50 | 7.90 | 39.3 | 36.7 | ..... | none | 0.512<br>0.512 | 5.19 | 6.91 |
| 7 | 1.0283 | 3.40 | 7.75 | 39.0 | 36.3 | 0.6936 | none | 0.502<br>0.502 | 7.04 | 8.73 |
| 9 | 1.0277 | 3.40 | 7.63 | 38.4 | 35.9 | 0.6724 | positive | 0.491<br>0.492 | 8.96 | 10.63 |
| 11 | 1.0273 | 3.30 | 7.47 | 38.0 | 35.5 | 0.6544 | positive | 0.482<br>0.483 | 10.65 | 12.27 |
| 13 | 1.0270 | 3.30 | 7.41 | .... | 35.1 | ..... | positive | 0.473<br>0.474 | 12.31 | 13.91* |
| 15 | 1.0262 | 3.20 | 7.18 | 37.0 | 34.8 | 0.6232 | positive | 0.465<br>0.466 | 13.79 | 15.36* |

* Irregularities in analytical results due probably to inexact preparation of sample or incomplete mixing.

that these samples were not accurately prepared or were imperfectly mixed before analysis. Owing to the fact that the freezing-point result on Sample B approaches the highest obtained on authentic samples of milk, a discrepancy is shown, as may be expected, between the two series of results tabulated in the last two columns. Results calculated on the basis of the freezing point of the original sample correspond closely with the known percentages of added water, whereas results calculated on the basis of the average freezing point of pure milk (−0.550°C.) show discrepancies varying from 2.72 to 3.18 per cent throughout the series. Results are in much closer agreement with actual composition in the series headed by Sample A, and the same is true in the series headed by Sample C with the exception of the irregularities pointed out in the last three mixtures.

The four series of mixtures included in Table 3 afford a more instructive and complete illustration of the relationships among the results obtained by the various methods applied. The series headed by Sample II-A exhibits a general resemblance to the series headed by Sample A included in Table 1, except that in the former series the percentages of added water as determined by the freezing tests are more closely in agreement with the mixtures of known composition. The series based on Sample II-C also exhibits resemblances to the Holstein series included in Table 1 except that the indications of added water based on the serum examinations are positive almost at the beginning. The two series based respectively on Samples II-B and II-D are also very similar. They are illustrative of a type of milk which exhibits striking contrasts

TABLE 3.

*Samples containing known percentages of added water.*

(Libby, McNeill & Libby Laboratory.)

| Added Water | Specific Gravity at 15.6°C. | Fat | Solids not Fat | Immersion Refractometer Readings 20°C. | | Ash in Acetic Serum (Gram in 100 cc.) | Added Water Indicated | Cryoscopic Determination | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Acetic Serum | Copper Serum | | | Freezing point | Added Water $\frac{(T-T')100}{T}$ | Added Water $\frac{(0.550-T')100}{0.550}$ |
| **Sample II-A.—Herd, 7 Holsteins.** | | | | | | | | | | |
| *per cent* | | *per cent* | *per cent* | | | | | −0°C. | *per cent* | *per cent* |
| None | 1.0320 | 3.07 | 8.61 | 42.22 | 38.45 | 0.7374 | None | 0.550<br>0.549 | None | None |
| 5 | 1.0305 | 2.90 | 8.21 | 40.92 | 37.39 | 0.7248 | None | 0.520<br>0.519 | 5.45 | 5.45 |
| 7 | 1.0300 | 2.85 | 8.07 | 40.30 | 36.78 | 0.7160 | None | 0.508<br>0.509 | 7.45 | 7.54 |
| 9 | 1.0294 | 2.78 | 7.91 | 40.00 | 36.30 | 0.7073 | None | 0.500<br>0.499 | 9.09 | 9.18 |
| 11 | 1.0288 | 2.75 | 7.75 | 39.25 | 35.78 | 0.6946 | Probable | 0.488<br>0.488 | 11.19 | 11.27 |
| 13 | 1.0281 | 2.70 | 7.56 | 38.61 | 35.41 | 0.6832 | Present | 0.476<br>0.477 | 13.27 | 13.36 |
| 15 | 1.0275 | 2.65 | 7.41 | 38.00 | 35.09 | ..... | Present | 0.466<br>0.466 | 15.19 | 15.27 |
| **Sample II-B.—Herd, 7 Pure-bred Jerseys.** | | | | | | | | | | |
| None | 1.0344 | 5.25 | 9.65 | 46.40 | 39.51 | 0.8172 | None | 0.554<br>0.554 | None | None |
| 5 | 1.0329 | 5.00 | 9.23 | 44.36 | 38.32 | 0.7672 | None | 0.525<br>0.525 | 5.32 | 4.54 |
| 7 | 1.0324 | 4.90 | 9.08 | 43.68 | 38.00 | ..... | None | 0.513<br>0.512 | 7.57 | 6.82 |
| 9 | 1.0317 | 4.83 | 8.89 | 43.21 | ..... | 0.7452 | None | 0.503<br>0.501 | 9.47 | 8.73 |
| 11 | 1.0312 | 4.65 | 8.73 | 42.40 | 37.38 | 0.7332 | None | 0.489<br>0.489 | 11.81 | 11.09 |
| 13 | 1.03 04 | 4.60 | 8.52 | 41.62 | 36.80 | 0.7208 | None | 0.475<br>0.475 | 14.34 | 13.26* |
| 15 | 1.0296 | 4.50 | 8.30 | 40.98 | 36.43 | 0.7184 | None | 0.466<br>0.466 | 15.93 | 15.27 |
| **Sample II-C.—Herd, 14 Grade Holsteins (P.M. Milkings, August 11-12, 1921).** | | | | | | | | | | |
| None | 1.0293 | 3.75 | 8.07 | 39.65 | 36.29 | 0.8036 | None | 0.538<br>0.538 | None | 2.18<br>2.18 |
| 5 | 1.0280 | 3.55 | 7.71 | 38.62 | 35.19 | 0.7672 | Probable | 0.508<br>0.508 | 5.58<br>5.58 | 7.64<br>7.64 |
| 7 | 1.0277 | 3.48 | 7.61 | 38.31 | 34.82 | 0.7572 | Present | 0.498<br>0.497 | 7.44<br>7.62 | 9.45<br>9.64 |
| 9 | 1.0272 | 3.40 | 7.48 | 37.57 | 34.40 | 0.7468 | Present | 0.487<br>0.487 | 9.48<br>9.48 | 11.45<br>11.45 |
| 11 | 1.0267 | 3.29 | 7.33 | 37.07 | 34.18 | 0.7192 | Present | 0.475<br>0.475 | 11.71<br>11.71 | 13.64<br>13.64 |
| 13 | 1.0261 | 3.23 | 7.17 | 36.64 | 33.57 | 0.7084 | Present | 0.465<br>0.465 | 13.57<br>13.57 | 15.45<br>15.45 |
| 15 | 1.0253 | 3.19 | 6.96 | 36.11 | 33.33 | 0.6904 | Present | 0.454<br>0.453 | 15.61<br>15.80 | 17.45<br>17.64 |

* Irregularities in analytical results due probably to inexact preparation of sample or incomplete mixing.

TABLE 3.—Continued.

*Samples containing known percentages of added water.*

| ADDED WATER | SPECIFIC GRAVITY AT 15.6°C. | FAT | SOLIDS NOT FAT | IMMERSION REFRACTOMETER READINGS 20°C. | | ASH IN ACETIC SERUM (Gram in 100 cc.) | ADDED WATER INDICATED | CRYOSCOPIC DETERMINATION | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Acetic Serum | Copper Serum | | | Freezing point | Added Water $\frac{(T-T')\,100}{T}$ | Added Water $\frac{(0.550-T')\,100}{0.550}$ |
| **Sample II-D.—Herd, 8 Pure-bred Jerseys (P.M. Milkings, August 24-25. 1921).** | | | | | | | | | | |
| *per cent* | | *per cent* | *per cent* | | | | | −0°C. | *per cent* | *per cent* |
| None | 1.0313 | 4.95 | 8.82 | 42.70 | 37.73 | 0.8268 | None | 0.547<br>0.546 | None | 0.55<br>0.73 |
| 5 | 1.0300 | 4.68 | 8.44 | 41.15 | 36.59 | 0.7906 | None | 0.519<br>0.518 | 5.10<br>5.13 | 5.64<br>5.82 |
| 7 | 1.0295 | 4.60 | 8.30 | 40.32 | 36.25 | 0.7792 | None | 0.504<br>0.504 | 7.86<br>7.69 | 8.36<br>8.36 |
| 9 | 1.0289 | 4.50 | 8.13 | 39.69 | 35.75 | 0.7620 | None | 0.493<br>0.493 | 9.87<br>9.71 | 10.36<br>10.36 |
| 11 | 1.0285 | 4.46 | 8.02 | 39.18 | 35.32 | 0.7456 | None | 0.482<br>0.481 | 11.88<br>11.90 | 12.36<br>12.54 |
| 13 | 1.0280 | 4.35 | 7.87 | 38.60 | 34.98 | 0.7244 | Probable | 0.473<br>0.473 | 13.53<br>13.37 | 14.00<br>14.00 |
| 15 | 1.0276 | 4.25 | 7.75 | 38.12 | 34.61 | 0.7112 | Present | 0.462<br>0.461 | 15.54<br>15.57 | 16.00<br>16.18 |

in comparison with milk obtained from Holstein herds. In the case of the series II-D, the refractometer readings taken in conjunction with the ash results on the acetic serum fail to yield positive indications of added water as high as 13 per cent, and in the case of II-B no positive indication of added water is observable in any of the mixtures. Doubtless the series could have been continued so as to include 19 or even 21 per cent of added water before results of the serum examinations could be interpreted as positive. Serious irregularities are apparent at the close of this latter series which seem to render doubtful the calculated percentages included at the bottom of the last two columns.

The general contrasts between the series based on Samples A, B, and C, (Table 2), and the series based on Samples II-A and II-C, (Table 3), on the one hand, and the series based on Sample II-B and II-D, (Table 3), on the other, may be anticipated after an inspection of the general results of the analyses, chiefly the results for fat-free solids. It will be noted that the figures for fat-free solids in the original Holstein samples range from 8.07 to 8.61, whereas, in the case of the Jersey herd samples, the fat-free solids figures are respectively 8.82 and 9.65 with the exception of the slight irregularities observed near the close of the series based on Sample II-B. The results derived from the freezing-point determinations are reasonably in agreement with the known composition of the mixtures. The discrepancies between the calculated and the known percentages range throughout the four series from a minimum of 0.09 to a maximum of 0.93, omitting the results obtained on the 13 per cent mixture in series II-B. Irregularities among results based on the freezing-point determinations may be due to various factors, among which are improperly prepared samples, gradual changes in acidity, inaccuracy in manipulation of the freezing-point tests, personal errors, etc. Nevertheless, in all series of samples added water is positively indicated in each mixture of known composition by means of the cryoscopic test, and the results are sufficiently consistent to justify the conclusion that the indications of added water are

TABLE 4.

*Milk containing known percentages of added water*.*

(Analysts, L. S. Palmer and R. D. Evans.)

| ADDED WATER | SPECIFIC GRAVITY AT 15.6°C. | FAT | SOLIDS NOT FAT | IMMERSION REFRACTOMETER READING 20°C. | | ADDED WATER INDICATED | FREEZING POINT | ADDED WATER $\frac{(T-T')100}{T}$ |
|---|---|---|---|---|---|---|---|---|
| | | | | Acetic Serum | Copper Serum | | | |
| *per cent* | | *per cent* | *per cent* | | | | –0°C. | *per cent* |
| Whole Milk | 1.0312 | 3.25 | 8.45 | 44.10 | 37.85 | None | 0.544 | .... |
| 5 | 1.0302 | 3.15 | 8.18 | 42.70 | 37.4 | None | 0.516 | 5.14 |
| 7 | 1.0296 | 3.00 | 8.0 | 41.72 | 37.00 | None | 0.505 | 7.11 |
| 9 | 1.0288 | 2.90 | 7.78 | 41.20 | 36.65 | None | 0.492 | 9.55 |
| 11 | 1.0284 | 2.80 | 7.66 | 40.90 | 36.3 | None | 0.482 | 11.21 |
| 13 | 1.0280 | 2.75 | 7.55 | 39.55 | 35.6 | Probable | .... | .... |
| 15 | 1.0274 | 2.70 | 7.39 | 39.50 | 35.5 | Probable | 0.467 | 14.15 |

* Cow: Ayrshire from dairy herd, University Farm, St. Paul, Minn.

TABLE 5.

*Market milks tested for added water.*

| NUMBER OF SAMPLE | SPECIFIC GRAVITY AT 15.6°C. | FAT | SOLIDS NOT FAT | IMMERSION REFRACTOMETER AT 20°C. | | ASH IN ACETIC SERUM (Gram in 100 cc.) | ADDED WATER INDICATED | CRYOSCOPIC EXAMINATION | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Acetic Serum | Copper Serum | | | Freezing Point | Added Water $\frac{(0.550-T')100}{0.550}$ |
| | | *per cent* | *per cent* | | | | | –0°C. | *per cent* |
| 6720 | 1.0294 | 3.4 | 8.17 | 40.00 | 36.51 | ..... | None | 0.530 | 3.64 |
| 6725 | 1.0300 | 3.5 | 8.34 | 40.23 | 37.37 | ..... | None | 0.546 | .... |
| 6793 | 1.0286 | 3.5 | 7.99 | 39.36 | 36.50 | 0.7548 | None | 0.546 | .... |
| 6902 | 1.0302 | 3.6 | 8.40 | 40.98 | ..... | 0.7380 | None | 0.516 | 6.18 |
| 6904 | 1.0302 | 4.0 | 8.49 | 41.66 | ..... | 0.7556 | None | 0.536 | .... |
| 6955 | 1.0298 | 3.9 | 8.38 | 39.66 | 36.25 | 0.7130 | None | 0.498 | 9.45 |
| 6963 | 1.0276 | 3.8 | 7.81 | 38.02 | 35.00 | 0.6012 | Present | 0.466 | 15.27 |
| 6983 | 1.0292 | 4.3 | 8.31 | 40.35 | 37.00 | 0.7348 | None | 0.533 | 3.09 |
| 7012 | 1.0296 | 3.5 | 8.25 | 39.38 | 36.18 | 0.7264 | | 0.535 | .... |
| 7152 | 1.0281 | 3.5 | 7.87 | 38.80 | 35.78 | 0.6800 | Present | 0.504 | 8.39 |

reliable, well below 1 per cent under the conditions exhibited by these mixtures. In other words, the cryoscopic method is dependable in all cases.

Results based on the average freezing point (−0.550°C.) for normal milk yield somewhat wider discrepancies but in the samples included in this investigation are well below 3 per cent. The examination of the various serums yields results which are obviously dependent to a great extent on the composition of the original milk. With reference to herds it may be concluded in general that milk obtained from Holsteins is more susceptible to positive indications as a result of serum examination than milk obtained from Jerseys or other breeds exhibiting similar characteristics. In certain cases, not necessarily extreme but commonly occurring in many localities, market milk may actually be highly mixed with water and yet yield no positive indication of the fact as a result of the refractometer readings or the ash determinations, whereas such adulteration is immediately apparent as a result of cryoscopic tests even to a figure as low as approximately 3 per cent. It is conceivable, in fact positively demonstrated in these results, that certain types of milk may contain as high as 20 per cent or even more of actual added water without yielding positive indications of adulteration by means of any heretofore applied method of examination, while, on the other hand, certain other types of milk, derived chiefly from Holstein sources, will yield positive results in cases of adulteration down to a much lower percentage.

These general conclusions are instructive and should be borne in mind in connection with the routine examination of market milk. Obviously no sample of milk should be condemned simply because it happens to yield on analysis a low specific gravity result or fat-free solids below 8 per cent. Neither can it safely be concluded that a sample is unsophisticated for the simple reason that it yields serum examination results falling within the accepted limits. Numerous market milk samples have been unjustifiably condemned as a result of the ordinary course of analysis, and it is also true that numerous samples have been passed or merely reported as suspicious as a result of analytical procedures based on prepared serums. Therefore, as a valuable adjunct to methods heretofore applied the cryoscopic examination serves a very useful and just purpose, for the reason that no sample of milk which yields a normal freezing-point result will be unfairly condemned and, on the other hand, no sample which as a result of general analysis is apparently normal but at the same time yields an abnormal freezing-point result can be reported upon favorably. Furthermore, the cryoscopic examination not only affords a positive indication of adulteration but also yields results in terms of percentages which may be regarded as reasonably accurate, allowing for tolerances warranted by an investigation under local conditions.

## GENERAL STATEMENT.

It is necessary to make clear what is to be comprehended under the title—*the cryoscopic method.* Essentially involved in this method are the following:

(1) The procedure for testing and correcting the thermometer.

(2) The procedure for making the freezing-point determination, strict attention being given to the following:

(a) The temperature of the cooling bath;

(b) the degree of supercooling; and

(c) a close adherence to other requirements, among which are quantity of sample, rate of stirring and the method of using the thermometer.

The above cryoscopic method does not, so far as can now be stipulated, involve any special design or type of apparatus, but it is important that all essential conditions be maintained uniform and that the instructions given in the procedure be strictly followed. In other words, the cryoscopic procedure, described in detail in the report of the referee for 1920[1] and subjected to collaborative study during the present year, is an attempt to standardize the method, and it is therefore understood that whatever may be the design or type of thermometer or cryoscope the outlined conditions are to be carefully observed.

## RECOMMENDATIONS.

Having in mind the above explanatory statement, it is recommended—

(1) That the cryoscopic method of examination of milk be adopted as an official method.

(2) That a continued study be made of the cryoscopic method both as applied to the examination of milk and also as applied as a general method for the examination of other food products.

# CRYOSCOPY OF MILK.

By E. M. Bailey (Agricultural Experiment Station, New Haven, Conn.), *Associate Referee.*

This report deals only with the freezing points of authentic samples of milk from individual cows and herds as outlined in the schedule of study for 1921, with certain additions thereto.

Two hundred sixteen samples are represented in the combined reports. Partial or complete analyses with freezing-point determinations, the latter largely in duplicate, were made. These were classified in appropriate groups and the results appear in tabular form in Tables 1, 2, 3 and 4.

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[1] *J. Assoc. Official Agr. Chemists*, 1921, 5: 172.

TABLE 1.

*Analyses and freezing-point depressions of authentic milk.*

(NORMAL INDIVIDUAL COWS.)

| HERD | INDIVIDUAL COW NO. OR SAMPLE NO. | BREED | DATE 1921 | SPECIFIC GRAVITY AT 15.6°C. | FAT | SOLIDS NOT FAT | LACTOSE | ASH | ACIDITY | FREEZING POINT | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Collaborator, Libby, McNeill & Libby* | | | | | | | | | | | |
| | | | | | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *−0° C.* | |
| H. T. | 4 B | Holstein | 9–14 P. M. | 1.0306 | 3.3 | 8.30 | 4.64 | 0.639 | 0.145 | 0.532 | 0.531 |
| H. A. | 5 B | | | 1.0310 | 3.6 | 8.47 | 3.71 | 0.799 | 0.147 | 0.551 | 0.551 |
| H. A. | 6 B | | | 1.0380 | 3.8 | 10.26 | 3.65 | 0.805 | 0.155 | 0.566 | 0.566 |
| B. J. F. | 7 B | | | 1.0300 | 3.3 | 8.15 | 4.39 | 0.660 | 0.140 | 0.533 | 0.533 |
| | 1 | Holstein, Grade | 9–18 P. M. | 1.0260 | 6.3 | 7.76 | 3.41 | 0.664 | 0.120 | 0.548 | 0.549 |
| | 7 | | | 1.0303 | 4.9 | 8.55 | 4.45 | 0.697 | 0.170 | 0.548 | 0.546 |
| | 8 | | | 1.0264 | 3.0 | 7.20 | 4.29 | 0.770 | 0.103 | 0.548 | 0.548 |
| | 9 | | | 1.0303 | 3.8 | 8.34 | 4.37 | 0.678 | 0.165 | 0.538 | 0.537 |
| | 11 | | | 1.0288 | 4.7 | 8.14 | 4.16 | 0.728 | 0.153 | 0.549 | 0.548 |
| B. J. F. | . . . | Holstein, Pure | 9–14 P. M. | 1.0300 | 2.2 | 7.90 | 4.38 | 0.693 | 0.140 | 0.535 | 0.535 |
| B. V. | . . . . | Holstein, Grade | 9–16 P. M. | 1.0287 | 0.9* | 7.36 | 4.15 | 0.719 | 0.100 | 0.538 | 0.537 |
| A. H. | . . . . | | 9–21 P. M. | 1.0357 | 3.3 | 10.01 | 4.17 | 0.784 | 0.190 | 0.548 | 0.548 |
| J. N. | . . . . | | | 1.0316 | 3.7 | 8.63 | 4.34 | 0.707 | 0.153 | 0.551 | 0.550 |
| B. F. H. | . . . . | | | 1.0338 | 4.1 | 9.26 | 4.15 | 0.770 | 0.165 | 0.549 | 0 548 |
| E. K. | . . . . | Jersey, Grade | | 1.0328 | 4.3 | 9.05 | 4.68 | 0.730 | 0.165 | 0.542 | 0.540 |
| J. H. | . . . . | | 9–26 A. M. | 1.0326 | 3.1 | 8.77 | 5.03 | 0.628 | 0.115 | 0.560 | 0.560 |
| | | | 9–26 P. M. | 1.0320 | 2.8 | 8.57 | 4.78 | 0.622 | 0.118 | 0.546 | 0.545 |
| | 5 | Brown Swiss | 9–18 P. M. | 1.0256 | 5.3 | 7.46 | 3.65 | 0.647 | 0.145 | 0.549 | 0.548 |
| A. M. | . . . . | Durham, Grade | 9–16 P. M. | 1.0310 | 3.5 | 8.44 | 4.55 | 0.661 | 0.145 | 0.533 | 0.533 |
| A. S. | . . . . | | | 1.0328 | 4.0 | 8.99 | 4.61 | 0.676 | 0.160 | 0.545 | 0.547 |
| G. T. | . . . . | | 9–26 A. M. | 1.0318 | 4.0 | 8.74 | 3.75 | 0.782 | 0.110 | 0.548 | 0.549 |
| | | | 9–26 P. M. | 1.0310 | 4.3 | 8.60 | 3.56 | 0.797 | 0.120 | 0.541 | 0.540 |
| A. D. | . . . . | Durham—S. | 9–26 A. M. | 1.0340 | 4.9 | 9.48 | 4.63 | 0.829 | 0.135 | 0.550 | 0.550 |
| | | Horn | 9–26 P. M. | 1.0340 | 4.4 | 9.37 | 4.52 | 0.832 | 0.123 | 0.548 | 0.547 |
| O. R. | . . . . | Short Horn— | 9–26 A. M. | 1.0325 | 4.6 | 9.05 | 3.63 | 0.636 | 0.083 | 0.540 | 0.539 |
| | | Holstein | 9–26 P. M. | 1.0326 | 4.2 | 8.96 | 3.43 | 0.661 | 0.080 | 0.540 | 0.540 |
| F. F. | . . . . | Red Pole | 9–23 A. M. | 1.0304 | 5.5 | 8.94 | 4.53 | 0.695 | 0.138 | 0.551 | 0.551 |
| *Collaborator, Minnesota Dairy and Food Department.* | | | | | | | | | | | |
| . . . . . | 1 H | Holstein, Reg. | 5–9 | 1.0295 | 4.7 | 8.47 | . . . . . | . . . . | . . . . | 0.560 | . . . . |
| . . . . . | 2 H. | | | 1.0281 | 4.2 | 8.02 | . . . . | . . . . . | . . . . . | 0.554 | . . . . |
| . . . . . | 3 H. | | | 1.0312 | 3.4 | 8.63 | . . . . | . . . . | . . . . . | 0.550 | . . . . . |
| . . . . . | 4 H. | | | 1.0313 | 2.9 | 8.54 | . . . . | . . . | . . . . | 0.542 | . . . . . |
| . . . . . | 5 H. | | | 1.0310 | 2.3 | 8.34 | . . . . . | . . . | . . . . | 0.540 | . . . . . |
| . . . . . | 6230 | Holstein | 5–14 | 1.0305 | 3.3 | 8.57 | . . | . | . . . | 0.544 | . . . . |
| . . . . . | 6231 | | | 1.0310 | 2.6 | 8.24 | . | . . . | . . . . | 0.541 | . . . . |
| . . . . . | 6232 | | | 1.0310 | 2.8 | 8.48 | . . . . | . . . | . . | 0.543 | . . . . . |
| . . . . . | 6233 | | | 1.0320 | 2.4 | 8.60 | . . . | . . | . . . . | 0.540 | . . . . . |
| . . . . . | 6234 | | | 1.0330 | 2.8 | 8.95 | . . . | . . . . | . . . . . | 0.554 | . . . . . |
| . . . . . | 6235 | | | 1.0310 | 2.6 | 8.43 | . . . | . . . . . | . . . . . | 0.556 | . . . . . |
| . . . . . | 6236 | | | 1.0315 | 4.9 | 9.07 | . . . | . . . . . | . . . . . | 0.552 | . . . . . |
| . . . . . | 6237 | | | 1.0330 | 3.3 | 9.05 | . . . | . . . | . . . . . | 0.540 | . . . . |
| . . . . . | 6238 | | | 1.0310 | 4.4 | 8.78 | . . . | . . . . . | . . . . . | 0.545 | . . . . . |
| . . . . . | 6250 | | 5–17 | 1.0290 | 3.7 | 9.25 | . . | . . . . . | . . . . . | 0.546 | . . . . |
| . . . . . | 7022 | Durham | 9–7 | 1.0323 | 4.4 | 9.10 | . . . | . . . . . | . . . . . | 0.554 | . . . . . |
| St.L.P. | . . . . | (Br. not given) | 8–25 | 1.0315 | 3.8 | 8.80 | . . | . . . . . | . . . . . | 0.551 | . . . . |

* A. M. sample following day tested 1.6% fat.

TABLE 1.—Continued.

*Analyses and freezing-point depressions of authentic milk.*

(NORMAL INDIVIDUAL COWS—Continued.)

| HERD | INDIVIDUAL COW NO. OR SAMPLE NO. | BREED | DATE 1921 | SPECIFIC GRAVITY AT 15.6°C. | FAT | SOLIDS NOT FAT | LACTOSE | ASH | ACIDITY | FREEZING POINT | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Collaborator, Connecticut Agricultural Experiment Station.* | | | | | | | | | | | |
| | | | | | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *-0°C.* | |
| Y | 1 | Holstein | 4–5 A. M. | 1.0323 | 3.6 | 8.80 | .... | ..... | 0.17 | 0.573 | 0.574* |
| | | | 4–13 A. M. | 1.0317 | 3.2 | 8.58 | .... | ..... | 0.15 | 0.558 | 0.559 |
| | | | 4–15 A. M. | 1.0330 | 3.3 | 8.92 | .... | ..... | 0.16 | 0.565 | 0.566 |
| | | | | 1.0330 | 3.3 | 8.92 | .... | ..... | 0.15 | 0.565 | 0.565 |
| | | | 4–16 A. M. | 1.0322 | 3.4 | 8.74 | .... | ..... | 0.16 | 0.565 | 0.565 |
| | | | 4–20 A. M. | 1.0315 | 3.3 | 8.55 | .... | ..... | 0.15 | 0.562 | 0.562 |
| | | | 4–20 P. M. | 1.0305 | 3.3 | 8.30 | .... | ..... | 0.14 | 0.542 | 0.542 |
| | | | 5–20 P. M. | 1.0299 | 3.4 | 8.17 | .... | ..... | 0.14 | 0.536 | 0.537 |
| | 2 | | 4–6 A. M. | 1.0320 | 4.3 | 8.88 | .... | ..... | 0.14 | 0.572 | 0.572* |
| | | | 4–13 A. M. | 1.0311 | 3.4 | 8.46 | .... | ..... | 0.15 | 0.544 | 0.544 |
| | | | 4–16 A. M. | 1.0317 | 4.1 | 8.76 | .... | ..... | 0.14 | 0.562 | 0.562 |
| | | | 4–20 A. M. | 1.0317 | 3.6 | 8.67 | .... | ..... | 0.13 | 0.553 | 0.554 |
| | | | 4–26 P. M. | 1.0307 | 3.8 | 8.46 | .... | ..... | 0.15 | 0.547 | 0.546 |
| | | | 5–18 P. M. | 1.0309 | 4.3 | 8.60 | .... | ..... | 0.13 | 0.551 | 0.552 |
| | 3 | | 4–7 A. M. | 1.0337 | 5.4 | 9.54 | .... | ..... | 0.15 | 0.572 | 0.572* |
| | | | 4–13 A. M. | 1.0321 | 4.7 | 8.98 | .... | ..... | 0.15 | 0.557 | 0.557 |
| | | | 4–20 A. M. | 1.0330 | 5.1 | 9.29 | .... | ..... | 0.15 | 0.562 | 0.563 |
| | | | 4–26 P. M. | 1.0325 | 5.0 | 9.14 | .... | ..... | 0.14 | 0.547 | 0.547 |
| | | | 5–20 P. M. | 1.0332 | 5.0 | 9.31 | .... | ..... | 0.15 | 0.549 | 0.550 |
| | 4 | | 4–9 A. M. | 1.0308 | 4.4 | 8.60 | .... | ..... | 0.12 | 0.560 | 0.560 |
| | | | 4–20 A. M. | 1.0320 | 3.6 | 8.73 | .... | ..... | 0.14 | 0.562 | 0.562 |
| | | | 4–9 P. M. | 1.0299 | 4.4 | 8.37 | .... | ..... | 0.12 | 0.552 | 0.550 |
| | | | 4–26 P. M. | 1.0308 | 3.1 | 8.34 | .... | ..... | 0.15 | 0.543 | 0.543 |
| | | | 5–26 P. M. | 1.0315 | 3.3 | 8.85 | .... | ..... | 0.13 | 0.543 | 0.543 |
| | 5 | | 4–8 A. M. | 1.0333 | 3.7 | 9.07 | .... | ..... | 0.15 | 0.552 | 0.552 |
| | 6 | | 4–16 A. M. | 1.0326 | 3.3 | 8.82 | .... | ..... | 0.12 | 0.571 | 0.571* |
| | | | 4–22 A. M. | 1.0313 | 3.5 | 8.52 | .... | ..... | 0.11 | 0.571 | 0.571 |
| | | | 4–25 P. M. | 1.0301 | 3.1 | 8.15 | .... | ..... | 0.11 | 0.533 | 0.534 |
| | | | 4–26 P. M. | 1.0309 | 3.7 | 8.48 | .... | ..... | 0.10 | 0.544 | 0.545 |
| | 7 | | 4–22 A. M. | 1.0322 | 4.0 | 8.85 | .... | ..... | 0.12 | 0.571 | 0.571* |
| | | | 4–25 P. M. | 1.0312 | 4.0 | 8.60 | .... | ..... | 0.11 | 0.545 | 0.546 |
| | | | 5–26 P. M. | 1.0326 | 4.3 | 9.03 | .... | ..... | 0.12 | 0.553 | 0.553 |
| | 8 | | 4–22 A. M. | 1.0342 | 4.6 | 9.48 | .... | ..... | 0.13 | 0.580 | 0.580* |
| | | | 4–25 P. M. | 1.0333 | 4.0 | 9.13 | .... | ..... | 0.12 | 0.542 | 0.542 |
| | | | 5–18 P. M. | 1.0343 | 5.0 | 9.58 | .... | ..... | 0.11 | 0.552 | 0.553 |
| | 9 | | 4–22 A. M. | 1.0340 | 3.8 | 9.28 | .... | ..... | 0.15 | 0.571 | 0.571* |
| | | | 4-25 P. M. | 1.0317 | 4.7 | 8.90 | .... | ..... | 0.12 | 0.543 | 0.544 |
| | | | 5–18 P. M. | 1.0318 | 4.6 | 8.94 | .... | ..... | 0.12 | 0.551 | 0.552 |
| | 10 | | 4–22 A. M. | 1.0320 | 4.1 | 8.83 | .... | ..... | 0.15 | 0.572 | 0.572* |
| | | | 5–18 P. M. | 1.0301 | 4.0 | 8.33 | .... | ..... | 0.12 | 0.542 | 0.542 |
| | 11 | | 4–22 A. M. | 1.0320 | 3.7 | 8.75 | .... | ..... | 0.15 | 0.562 | 0.562 |
| | | | 5–18 P. M. | 1.0303 | 3.9 | 8.35 | .... | ..... | 0.15 | ..... | 0.543 |

* See discussion.

TABLE 1.—Continued.

*Analyses and freezing-point depressions of authentic milk.*

(NORMAL INDIVIDUAL COWS—Continued.)

| HERD | INDIVIDUAL COW NO. OR SAMPLE NO. | BREED | DATE 1921 | SPECIFIC GRAVITY AT 15.6°C. | FAT | SOLIDS NOT FAT | LACTOSE | ASH | ACIDITY | FREEZING POINT | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Collaborator, Connecticut Agricultural Experiment Station—Continued.* | | | | | | | | | | | |
| | | | | | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | -0°C. | |
| Y | 12 | Holstein | 4–23 A. M. | 1.0301 | 6.8 | 8.88 | .... | ..... | 0.16 | 0.572 | 0.572* |
| | | | 5–18 P. M. | 1.0327 | 6.5 | 9.47 | .... | ..... | 0.15 | ..... | 0.551 |
| | 13 | | 4–23 A. M. | 1.0304 | 3.8 | 8.37 | .... | ..... | 0.12 | 0.540 | 0.540 |
| | | | 5–26 P. M. | 1.0316 | 4.6 | 8.84 | .... | ..... | 0.12 | ..... | 0.542 |
| | 14 | | 4–23 A. M. | 1.0319 | 3.8 | 8.75 | .... | ..... | 0.16 | 0.549 | 0.549 |
| | | | 5–20 P. M. | 1.0320 | 4.0 | 8.81 | .... | ..... | 0.16 | 0.539 | 0.538 |
| | 15 | | 4–27 A. M. | 1.0317 | 3.5 | 8.65 | ... | .... | 0.11 | 0.561 | 0.561* |
| | | | 4–25 P. M. | 1.0298 | 3.0 | 8.76 | .... | ..... | 0.13 | 0.523 | 0.523 |
| | | | 4–26 P. M. | 1.0305 | 3.3 | 8.30 | .... | ..... | 0.14 | 0.532 | 0.532 |
| | | | 4–27 P. M. | 1.0301 | 3.2 | 8.17 | .... | ..... | 0.13 | 0.536 | 0.537 |
| | | | 5–20 P. M. | 1.0312 | 3.4 | 8.48 | ... | ..... | 0.13 | 0.544 | 0.545 |
| | 16 | | 4–26 P. M. | 1.0302 | 4.5 | 8.45 | .... | .... | 0.16 | 0.540 | 0.541 |
| | | | 5–20 P. M. | 1.0317 | 4.6 | 8.87 | .... | ..... | 0.16 | ..... | 0.542 |
| | 17 | | 4–26 P. M. | 1.0294 | 3.5 | 8.06 | .... | ..... | 0.13 | 0.541 | 0.541 |
| | | | 5–20 P. M. | 1.0291 | 3.5 | 7.98 | .... | ..... | 0.12 | ..... | 0.542 |
| F. | 1 | | 4–16 A. M. | 1.0310 | 3.2 | 8.40 | .... | ..... | 0.14 | 0.542 | 0.542 |
| | | | 5–6 P. M. | 1.0304 | 3.1 | 8.23 | .... | ..... | 0.14 | 0.541 | 0.541 |
| | 2 | | 4–18 A. M. | 1.0313 | 2.8 | 8.38 | .... | ..... | 0.15 | 0.542 | 0.542 |
| | | | 5–5 P. M. | 1.0295 | .... | .... | .... | ..... | 0.14 | 0.543 | 0.544 |
| | 3 | | 4–18 A. M. | 1.0329 | .... | .... | .... | ..... | 0.14 | 0.550 | 0.551 |
| | | | 5–5 P. M. | 1.0296 | 3.2 | 8.05 | .... | ..... | 0.14 | 0.532 | 0.533 |
| | 4 | | 4–18 A. M. | 1.0308 | 3.1 | 8.34 | .... | ..... | 0.15 | 0.542 | 0.543 |
| | | | 5–5 P. M. | 1.0301 | 3.2 | 8.17 | .... | ..... | 0.15 | 0.529 | 0.530 |
| | 5 | | 4–18 A. M. | 1.0318 | 3.4 | 8.64 | .... | .. | 0.16 | 0.543 | 0.543 |
| | | | 5–5 P. M. | 1.0317 | 3.5 | 8.64 | .... | ..... | 0.16 | 0.542 | 0.540 |
| | 6 | | 4–19 A. M. | 1.0280 | 4.0 | 7.81 | .... | ..... | 0.11 | 0.545 | 0.546 |
| | | | 5–5 P. M. | 1.0307 | 4.0 | 8.50 | .... | ..... | 0.13 | 0.541 | 0.542 |
| | 7 | | 4–19 A. M. | 1.0323 | 4.1 | 8.90 | .... | ..... | 0.14 | 0.552 | 0.552 |
| | | | 5–6 P. M. | ..... | 3.5 | .... | .... | ..... | 0.11 | 0.542 | 0.542 |
| | 8 | | 4–19 A. M. | 1.0335 | 4.8 | 9.35 | .... | .... | 0.14 | 0.548 | 0.549 |
| | | | 5–6 P. M. | 1.0310 | 2.9 | 8.33 | .... | ..... | 0.16 | 0.536 | 0.536 |
| | 9 | | 4–19 A. M. | 1.0298 | 3.8 | 8.23 | .... | ..... | 0.15 | 0.542 | 0.542 |
| | | | 5–6 P. M. | 1.0305 | 3.8 | 8.40 | .... | ..... | 0.14 | 0.534 | 0.534 |
| | 10 | | 4–19 A. M. | 1.0309 | 3.8 | 8.50 | .... | ..... | 0.13 | 0.547 | 0.547 |
| | | | 5–6 P. M. | 1.0310 | 5.1 | 8.79 | .... | ..... | 0.12 | 0.543 | 0.543 |
| | 11 | | 4–19 A. M. | 1.0301 | 3.5 | 8.23 | .... | ..... | 0.16 | 0.542 | 0.542 |
| | | | 5–6 P. M. | 1.0303 | 2.7 | 8.10 | .... | ..... | 0.17 | 0.535 | 0.535 |

* See discussion.

TABLE 1.—Concluded.

*Analyses and freezing-point depressions of authentic milk.*

(NORMAL INDIVIDUAL COWS—Concluded.)

| HERD | INDIVIDUAL COW NO. OR SAMPLE NO. | BREED | DATE 1921 | SPECIFIC GRAVITY AT 15.6°C. | FAT | SOLIDS NOT FAT | LACTOSE | ASH | ACIDITY | FREEZING POINT | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Collaborator, Connecticut Agricultural Experiment Station—Concluded.* | | | | | | | | | | | |
| | | | | | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | -0°C. | |
| S | 1 | Jersey | 4–29 A. M. | 1.0336 | 6.0 | 9.63 | .... | ..... | 0.17 | 0.542 | 0.543 |
| | 2 | Ayrshire | 4–29 A. M. | 1.0309 | 4.1 | 8.56 | .... | ..... | 0.18 | 0.531 | 0.532 |
| | 3 | Guernsey | 4–29 A. M. | 1.0309 | 5.1 | 8.77 | .... | ..... | 0.20 | 0.549 | 0.549 |
| P | 5 | (Br. not given) | 5–11 P. M. | 1.0320 | 3.7 | 8.75 | .... | ..... | 0.16 | 0.532 | 0.533 |
| | | | 7–6 P. M. | 1.0303 | 4.7 | 8.53 | .... | ..... | 0.14 | 0.535 | 0.536 |
| | 6 | (Br. not given) | 5–11 P. M. | 1.0309 | .... | .... | .... | ..... | 0.09 | 0.542 | 0.542 |
| | | | 7–6 P. M. | 1.0271 | 3.8 | 7.55 | .... | ..... | 0.09 | 0.535 | 0.535 |

TABLE 2.

*Analyses and freezing-point depressions of authentic milk.*

(NORMAL HERDS.)

| HERD | NO. OF COWS | BREED | DATE 1921 | SPECIFIC GRAVITY AT 15.6°C. | FAT | SOLIDS NOT FAT | LACTOSE | ASH | ACIDITY | FREEZING POINT | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Collaborator, Libby, McNeill & Libby.* | | | | | | | | | | | |
| | | | | | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | -0°C. | |
| E. M. | 4 | Holstein, Grade | 4–1 P. M. | 1.0314 | 3.6 | 8.56 | .... | ..... | 0.145 | 0.535 | 0.534 |
| W. T. | 12 | ..... | 4–1 P. M. | 1.0305 | 2.8 | 8.17 | .... | ..... | 0.145 | 0.530 | 0.531 |
| W. T. | 13 | ..... | 6–22 P. M. | 1.0300 | 3.1 | 8.13 | 4.46 | ..... | 0.140 | 0.541 | 0.541 |
| W. T. | 15 | ..... | 7–14 P. M. | 1.0304 | 3.3 | 8.26 | .... | ..... | 0.135 | 0.538 | 0.538 |
| G. H. | 13 | ..... | 3–29 P. M. | 1.0290 | 3.6 | 7.98 | .... | ..... | 0.135 | 0.532 | 0.532 |
| G. H. | 13 | ..... | 7–14 P. M. | 1.0295 | 3.3 | 8.03 | 4.58 | ..... | 0.135 | 0.541 | 0.541 |
| W. B. | 6 | ..... | 4–1 P. M. | 1.0324 | 2.7 | 8.63 | .... | ..... | 0.150 | 0.529 | 0.530 |
| W. B. | 13 | Holstein, Grade and pure. | 6–22 P. M. | 1.0310 | 3.0 | 8.36 | 4.72 | ..... | 0.135 | 0.539 | 0.537 |
| W. B. | 13 | ..... | 7–14 P. M. | 1.0295 | 3.2 | 8.01 | .... | ..... | 0.130 | 0.533 | 0.532 |
| A. G. | 15 | Holstein, Pure | 8–2 P. M. | 1.0294 | 3.4 | 8.02 | .... | ..... | 0.135 | 0.538 | 0.537 |
| M. B. | 16 | Holstein, Grade | 9–16 P. M. | 1.0313 | 3.5 | 8.53 | 4.44 | 0.722 | 0.150 | 0.547 | 0.548 |
| H. W. | 11 | ..... | 9–21 P. M. | 1.0323 | 3.2 | 8.72 | 4.18 | 0.734 | 0.143 | 0.552 | 0.552 |
| O. R. | 10 | ..... | 9–26 A. M. | 1.0292 | 4.3 | 8.16 | 4.29 | 0.678 | 0.123 | 0.534 | 0.532 |
| | | | 9–26 P. M. | 1.0285 | 4.3 | 7.98 | 3.69 | 0.710 | 0.110 | 0.532 | 0.533 |
| G. P. | 6 | ..... | 9–21 P. M. | 1.0313 | 3.5 | 8.53 | 4.23 | 0.696 | 0.135 | 0.551 | 0.551 |
| D. F. M. 1 B. | 3 | Holstein, Pure | 9–14 P. M. | 1.0336 | 3.4 | 9.08 | 4.53 | 0.693 | 0.145 | 0.550 | 0.550 |
| B. J. F. 3 B. | 2 | ..... | 9–14 P. M. | 1.0300 | 2.7 | 8.05 | 4.39 | 0.676 | 0.145 | 0.535 | 0.535 |
| S. W. | 2 | Holstein-Jersey | 9–16 P. M. | 1.0313 | 3.7 | 8.57 | 4.61 | 0.684 | 0.155 | 0.549 | 0.548 |

TABLE 2.—Concluded.

*Analyses and freezing-point depressions of authentic milk.*

(NORMAL HERDS—Concluded.)

| HERD | NO. OF COWS | BREED | DATE 1921 | SPECIFIC GRAVITY AT 15.6°C. | FAT | SOLIDS NOT FAT | LACTOSE | ASH | ACIDITY | FREEZING POINT | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Collaborator, Libby, McNeill & Libby—Concluded.* | | | | | | | | | | | |
| | | | | | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *−0°C.* | |
| B. V. | 11 | Holstein 10, Jersey 1 | 9–16 P. M. | 1.0301 | 3.1 | 8.15 | 4.47 | 0.686 | 0.145 | 0.550 | 0.549 |
| A. P. T. | 10 | Holstein 4, Jersey 1, Black Pole 1, Durham 2, Hereford 1, Guernsey 1 | 9–21 P. M. | 1.0319 | 3.5 | 8.68 | 4.42 | 0.710 | 0.140 | 0.546 | 0.545 |
| E. K. | 12 | Holstein 2, Jersey 10 | 9–21 P. M. | 1.0320 | 3.8 | 8.76 | 4.52 | 0.704 | 0.148 | 0.543 | 0.542 |
| F. F. | 9 | Holstein 7, Red Pole 1, Jersey 1 | 9–23 A. M. | 1.0309 | 4.2 | 8.56 | 4.59 | 0.666 | 0.148 | 0.547 | 0.545 |
| D. L. | 2 | Jersey, Grade | 6–22 P. M. | 1.0329 | 5.4 | 9.30 | 4.92 | ..... | 0.142 | 0.550 | 0.549 |
| J. J. R. | 2 | ..... | 8–1 P. M. | 1.0316 | 3.3 | 8.56 | 4.82 | ..... | 0.130 | 0.551 | 0.551 |
| R. N. | 6 | Jersey 1, Short Horn 5 | 9–21 P. M. | 1.0316 | 3.8 | 8.66 | 4.67 | 0.672 | 0.150 | 0.550 | 0.550 |
| B. | 10 | Durham, Grade | 3–29 P. M. | 1.0321 | 3.8 | 8.77 | .... | ..... | 0.143 | 0.542 | 0.541 |
| A. D. | 6 | Durham–Short Horn | 9–26 A. M. | 1.0325 | 4.6 | 9.05 | 4.52 | 0.687 | 0.105 | 0.540 | 0.539 |
| | | | 9–26 P. M. | 1.0325 | 4.2 | 8.96 | 4.50 | 0.700 | 0.123 | 0.540 | 0.540 |
| P. W. | 7 | Reds, Grade | 6–20 P. M. | 1.0322 | 3.9 | 8.83 | 4.68 | ..... | 0.157 | 0.531 | 0.531 |
| P. W. | 7 | ..... | 7–14 P. M. | 1.0324 | 3.7 | 8.84 | .... | ..... | 0.130 | 0.534 | 0.533 |
| B. L. | 4 | Short Horn, Grade | 9–23 A. M. | 1.0314 | 3.6 | 8.57 | 4.48 | 0.706 | 0.150 | 0.557 | 0.556 |
| B. W. | 16 | Mixed Grade | 6–20 P. M. | 1.0326 | 3.7 | 8.98 | 4.45 | ..... | 0.152 | 0.536 | 0.537 |
| J. H. | 11 | ..... | 9–26 A. M. | 1.0318 | 4.0 | 8.74 | 4.68 | 0.696 | 0.118 | 0.548 | 0.549 |
| | | | 9–26 P. M. | 1.0310 | 4.3 | 8.60 | 4.55 | 0.698 | 0.120 | 0.541 | 0.540 |
| G. T. | 5 | ..... | 9–26 A. M. | 1.0313 | 4.5 | 8.73 | 4.29 | 0.719 | 0.128 | 0.552 | 0.553 |
| | | | 9–26 P. M. | 1.0313 | 4.1 | 8.65 | 4.14 | 0.750 | 0.125 | 0.552 | 0.553 |
| Factory Sample | 2300 | Mixed | 9–23 A. M. & P. M. | 1.0310 | 3.7 | 8.49 | 4.45 | 0.704 | 0.145 | 0.541 | 0.540 |
| *Collaborator, Connecticut Agricultural Experiment Station.* | | | | | | | | | | | |
| Y | 17 | Holstein | 4–27 A. M. | 1.0317 | 4.1 | 8.77 | .... | ..... | 0.13 | 0.553 | 0.553 |
| | | | 4–27 P. M. | 1.0309 | 3.5 | 8.44 | .... | ..... | 0.14 | 0.539 | 0.540 |
| F. | 11 | ..... | 5–2 A. M. | 1.0312 | 3.3 | 8.46 | .... | ..... | 0.16 | 0.559 | 0.560 |
| | | | 4–13 P. M. | 1.0313 | 3.7 | 8.56 | .... | ..... | 0.15 | 0.535 | 0.535 |
| | | | 4–18 P. M. | 1.0309 | 3.7 | 8.48 | .... | ..... | 0.16 | 0.539 | 0.540 |
| | | | 4–19 P. M. | 1.0310 | 3.4 | 8.44 | .... | ..... | 0.15 | 0.540 | 0.540 |
| | | | 5–2 P. M. | 1.0305 | 3.7 | 8.38 | ... | ..... | 0.14 | 0.540 | 0.540 |
| S. | 7 | ..... | 4–18 | ..... | 3.4 | .... | .... | ..... | 0.13 | 0.542 | 0.542 |
| B. | 7 | ..... | 4–18 | ..... | 3.5 | .... | .... | ..... | 0.13 | 0.550 | 0.550 |

TABLE 3.

*Analyses and freezing-point depressions of authentic milk.*

(Healthy cows under abnormal conditions of daily routine or environment.)

| HERD | DATE OF MILKING, 1921 | DESCRIPTION | SPECIFIC GRAVITY AT 15.6°C. | FAT | SOLIDS NOT FAT | LACTOSE | ASH | ACIDITY | FREEZING POINT | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | *Collaborator, Libby, McNeill & Libby.* | | | | | | | | |
| | | | | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | –0°C. | |
| | | *Individual Cows.* | | | | | | | | |
| A. M., 2A | 8–30 P. M. | Pure Jersey, driven 4 blocks to Fair, milking delayed 1 hour.. | 1.0327 | 7.6 | 9.69 | 4.29 | 0.817 | * | 0.563 | 0.563 |
| 2B | 9–14 P. M. | Same cow, normal conditions..... | 1.0398 | 7.0 | 11.34 | 1.80 | 0.874 | 0.160 | 0.562 | 0.563 |
| H. T., 4A | 8–30 P. M. | Pure Holstein, driven 6 miles to Fair, milking delayed 3½ hours.... | 1.0294 | 4.1 | 8.18 | 4.52 | 0.666 | * | 0.547 | 0.546 |
| 4B | 9–14 P. M. | Same cow, normal conditions ..... | 1.0306 | 3.3 | 8.30 | 4.64 | 0.639 | 0.145 | 0.532 | 0.531 |
| H. A., 5A | 8–31 P. M. | Pure Holstein, milking delayed 10½ hours... | 1.0322 | 3.9 | 8.84 | 4.00 | 0.815 | * | 0.578 | 0.577 |
| 5B | 9–14 P. M. | Same cow, normal conditions..... | 1.0310 | 3.6 | 8.47 | 3.71 | 0.799 | 0.147 | 0.551 | 0.551 |
| H. A.. 6A | 8–31 P. M. | Pure Holstein, milking delayed 10½ hours... | 1.0358 | 5.5 | 10.05 | 4.25 | 0.808 | * | 0.563 | 0.562 |
| 6B | 9–14 P. M. | Same cow, normal conditions..... | 1.0380 | 3.8 | 10.26 | 3.65 | 0.805 | 0.155 | 0.566 | 0.566 |
| B. J. F. 7A. | 8–31 P. M. | Pure Holstein, milking delayed 9½ hours.... | 1.0270 | 3.8 | 7.51 | 3.67 | 0.732 | * | 0.537 | 0.537 |
| 7B | 9–14 P. M. | Same cow, normal conditions..... | 1.0300 | 3.3 | 8.15 | 4.39 | 0.660 | 0.140 | 0.533 | 0.533 |
| | | *Herds.* | | | | | | | | |
| D.F.M. 1A | 8–30 P. M. | Three pure Holsteins, driven 6 miles to Fair, arriving 7:30 A. M. Sample taken from evening milking..... | 1.0318 | 5.48 | 9.05 | 4.59 | 0.762 | * | 0.571 | 0.571 |
| 1B | 9–14 P. M. | Same herd, normal conditions..... | 1.0336 | 3.40 | 9.08 | 4.53 | 0.693 | 0.145 | 0.550 | 0.550 |
| B. J. F. 3A | 8–30 P. M. | Two pure Holsteins, driven 5 miles to Fair, arriving 7:30 A. M. Sample taken from evening milking..... | 1.0316 | 3.3 | 8.55 | 4.59 | 0.700 | * | 0.561 | 0.560 |
| 3B | 9–14 P. M. | Same herd, normal conditions..... | 1.0300 | 2.7 | 8.05 | 4.39 | 0.676 | 0.145 | 0.535 | 0.535 |

* Freezing point observed same evening that samples were taken.

TABLE 4.

*Analyses and freezing-point depressions of authentic milk.*

(Cows diseased or otherwise abnormal physically.)

| HERD OR NO. | DATE OF MILKING, 1921 | DESCRIPTION | SPECIFIC GRAVITY AT 15.6°C. | FAT | SOLIDS NOT FAT | LACTOSE | ASH | ACIDITY | FREEZING POINT | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | *Collaborator, Libby, McNeill & Libby.* | | | | | | | | |
| | | *Individual Cows.* | | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | -0°C. | |
| 2 | 9–18 P. M. | Pure Holstein, tubercular reactor | 1.0263 | 4.0 | 7.38 | 3.10 | 0.763 | 0.137 | 0.550 | 0.549 |
| 3 | 9–19 A. M. | Grade Holstein, tubercular reactor, not milked evening of previous day | 1.0265 | 3.3 | 7.28 | 2.41 | 0.790 | 0.100 | 0.555 | 0.556 |
| 6 | 9–18 P. M. | Grade Holstein, tubercular reactor | 1.0298 | 3.4 | 8.13 | 2.41 | 0.731 | 0.130 | 0.560 | 0.560 |
| 12 | 9–18 P. M. | Grade Holstein, tubercular reactor | 1.0286 | 4.5 | 8.05 | 4.04 | 0.698 | 0.170 | 0.544 | 0.543 |
| 13 | 9–18 P. M. | Pure Holstein, tubercular reactor | 1.0280 | 4.6 | 7.92 | 4.17 | 0.661 | 0.155 | 0.550 | 0.550 |
| 14 | 9–18 P. M. | Pure Holstein, tubercular reactor | 1.0240 | 3.2 | 6.64 | 3.12 | 0.697 | 0.090 | 0.536 | 0.535 |
| 15 | 9–19 A. M. | Pure Holstein, tubercular reactor | 1.0364 | 3.7 | 7.34 | 2.80 | 0.782 | 0.100 | 0.551 | 0.549 |
| 4 | 9–18 P. M. | Guernsey-Holstein, tubercular reactor | 1.0299 | 4.7 | 8.42 | 2.67 | 0.714 | 0.145 | 0.550 | 0.552 |
| 10 | 9–18 P. M. | Grade Guernsey, tubercular reactor | 1.0270 | 4.6 | 7.67 | 2.96 | 0.767 | 0.136 | 0.542 | 0.543 |
| S C | 7–13 A. M. | Grade Jersey, poor physical condition | 1.0286 | 3.6 | 7.91 | .... | ..... | 0.123 | 0.522 | 0.523 |
| ..... | 7–14 A. M. | Symptoms of tuberculosis | ..... | .... | ..... | .... | ..... | 0.132 | 0.523 | 0.523 |
| A. M. 2B | 9–14 P. M. | Jersey, colostrum milk | 1.0398 | 7.0 | 11.34 | 1.80 | 0.874 | 0.160 | 0.562 | 0.563 |
| | | *Herd.* | | | | | | | | |
| ..... | 9–18 P. M. | 14 cows, mixed breeds, 8 tubercular | 1.0272 | 4.2 | 7.64 | 3.65 | 0.716 | 0.133 | 0.548 | 0.548 |
| | | *Collaborator, Connecticut Agricultural Experiment Station.* | | | | | | | | |
| P 1 | 5–11 P. M. | Holstein, tubercular reactor | 1.0300 | 3.7 | 8.25 | ... | ..... | 0.14 | 0.522 | 0.522 |
| | 7–6 P. M. | | 1.0272 | 4.4 | 7.69 | .... | ..... | 0.12 | 0.537 | 0.538 |
| P 2 | 5–11 P. M. | Holstein, tubercular reactor | 1.0304 | 2.0 | 8.00 | .... | ..... | 0.14 | 0.519 | 0.520 |
| | 7–6 P. M. | | 1.0215 | 9.5 | 7.38 | .... | ..... | 0.13 | 0.534 | 0.535 |
| P 3 | 5–11 P. M. | Holstein, tubercular reactor | 1.0287 | .... | ..... | .... | ..... | 0.12 | 0.522 | 0.522 |
| | 7–6 P. M. | | 1.0286 | 4.0 | 7.96 | .... | ..... | 0.18 | 0.550 | 0.552 |
| P 4 | 5–11 P. M. | Holstein, tubercular reactor | 1.0304 | 4.0 | 8.41 | . | .. . | 0.15 | 0.537 | 0.538 |
| | 7–6 P. M. | Holstein | 1.0277 | 4.2 | 7.78 | .. | ..... | 0.13 | 0.541 | 0.541 |

TABLE 4.—Concluded.

*Analyses and freezing-point depressions of authentic milk.*

(Cows diseased or otherwise abnormal physically.)

| HERD OR NO. | DATE OF MILKING, 1921 | DESCRIPTION | SPECIFIC GRAVITY AT 15.6°C. | FAT | SOLIDS NOT FAT | LACTOSE | ASH | ACIDITY | FREEZING POINT |
|---|---|---|---|---|---|---|---|---|---|
| | | *Collaborator, Minnesota State Dairy and Food Department.* | | | | | | | |
| | | | | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *-0°C.* |
| 517 | 9–22 | Heifer, 2 years old, in heat, tubercular reactor | 1.0301 | 3.4 | 8.35 | .... | ..... | ..... | 0.551 |
| 518 | 9–22 | Tubercular reactor, 10 years old | 1.0285 | 2.9 | 7.84 | .... | ..... | ..... | 0.541 ..... |
| 519 | 9–22 | Heifer, 3 years old, tubercular reactor | 1.0284 | 3.8 | 8.00 | .... | ..... | ..... | 0.548 ..... |
| 520 | 9–22 | Tubercular reactor | 1.0295 | 3.6 | 8.23 | .... | ..... | ..... | 0.544 ..... |
| 521 | 9–22 | Tubercular reactor, wild cow, one eye blind | 1.0328 | 3.6 | 9.05 | .... | ..... | ..... | 0.547 ..... |
| 522 | 9–22 | Tubercular reactor, produced 30 pounds of 80% butter in 7 consecutive days | 1.0293 | 3.1 | 8.08 | .... | ..... | ..... | 0.543 ..... |
| 523 | 9–22 | Tubercular reactor | 1.0303 | 4.4 | 8.60 | .... | ..... | ..... | 0.543 ..... |
| 524 | 9–22 | Tubercular reactor | 1.0328 | 3.3 | 9.00 | .... | ..... | ..... | 0.534 ..... |
| 525 | 9–22 | Tubercular cow with infected udder | 1.0254 | 0.5 | 6.59 | .... | ..... | ..... | 0.586 ..... |
| 526 | 9–22 | Tubercular reactor | 1.0290 | 3.7 | 8.23 | .... | ..... | ..... | 0.540 ..... |
| 513 | 9–17 | Holstein, calved 7 days previous to sampling, infected, still discharges pus | 1.0294 | 4.5 | 8.40 | .... | ..... | ..... | 0.554 ..... |
| 514 | 9–17 | Guernsey, abortion 11 days previous to sampling | 1.0341 | 4.3 | 9.53 | .... | ..... | ..... | 0.538 ..... |

## DISCUSSION OF RESULTS.

The term "normal" as applied to an individual cow or herd, is used to refer to animals which, in the judgment of the usual observer or dairyman, would be classed as healthy and which are fed and kept with ordinary care. It does not refer to animals which have been subject to clinical tests and pronounced sound by expert authority. The milk from animals which conform to this interpretation of the term is presumed to be normal milk.

Before taking up the discussion of freezing-point range, as shown by the combined data, attention is directed to certain extreme results which lie outside the experience of the collaborators as a whole. This refers to results below −0.570°C. found for a number of morning samples and to one extremely high figure, −0.523°C. observed in one case of evening milk from Holstein cows, Herd Y, summarized from Table 1, as follows:

TABLE 5.

*Results requiring further corroboration.*

| COW NO. | DATE | FREEZING POINT, --0°C |
|---|---|---|
| 1 | 4–5 A. M. | 0.573* |
| 2 | 4–6 A. M. | 0.572 |
| 3 | 4–7 A. M. | 0.572 |
| 6 | 4–16 A. M. | 0.571 |
| | 4-22 A. M. | 0.571 |
| 7 | 4–22 A. M. | 0.571 |
| 8 | 4–22 A. M. | 0.580 |
| 9 | 4–22 A. M. | 0.571 |
| 10 | 4–22 A. M. | 0.572 |
| 12 | 4–23 A. M. | 0.572 |
| 15 | 4–25 P. M. | 0.523 |

* Only instance of difference in the two observations made in each case; second showed 0.574.

The results shown in Table 5 were obtained in the laboratory of the writer who can vouch for the care with which the freezing-point observations were made and who can obtain no information of any abnormal conditions prevailing in the herd at the time the samples were taken. Although the low figures seem to be substantiated by their occurrence in several different cows, at intervals of from one to eighteen days, and in one case by recurrence in the same cow after an interval of six days, they were not duplicated or very closely approximated in any other samples from the same cows. Similarly, the very high figure obtained in the sample from No. 15 was not observed again in the examination of three other samples. There is no information upon which these samples can be declared abnormal in the sense of the term herein defined, yet for the reason that all the figures were obtained from one herd and because they have not been duplicated by the experience of any other collaborator or satisfactorily substantiated in the laboratory where they were obtained, they are recorded here with the provision that they require further corroboration and are classed accordingly.

As the data showing the relation between morning and evening milk both of individual cows and of herds as regards freezing-point depressions (Table 6), were submitted chiefly by one collaborator, no adequate comparison upon this point can be made.

The results reported from Connecticut show quite consistently greater depressions in the case of morning samples, whether from individual cows or herds. The results reported from the laboratory of Libby, McNeill and Libby do not entirely confirm this experience, but they are not complete enough to contradict it conclusively.

The extreme figures given in Table 6 show that the differences, without reference to their magnitude, are in the same general direction as the majority of other differences.

The last four observations in each group of Table 6 are from the Libby, McNeill and Libby laboratories. In the case of individual cows the data harmonize with those reported from Connecticut, but in the case of herds they do not. However, taking the six observations upon herd milk as they stand, the average freezing-point depression is 0.007°C. greater in the morning milk, an increase well beyond the limit of experimental error, and one which may be regarded as a real value.

The data showing the variation in freezing-point depressions from day to day, though not extensive enough to be conclusive, indicate that the variation between the morning milk and evening milk is greater than the variation between morning samples or evening samples on successive days.

TABLE 6.

*Variation in freezing-point depressions of morning and evening milk.*

| HERD AND COW NO. | NO. OF SAMPLES | | FREEZING POINT* A. M. | FREEZING POINT* P. M. | A. M. FREEZING POINT LOWER (+) OR HIGHER (−) THAN P. M. |
|---|---|---|---|---|---|
| | A. M. | P. M. | | | |
| | *Individual Cows* | | —0°C. | —0°C. | —0°C. |
| Y 1 | 5 | 2 | 0.563 | 0.539 | +0.024 |
| 2 | 3 | 2 | 0.553 | 0.549 | +0.004 |
| 3 | 2 | 2 | 0.560 | 0.548 | +0.012 |
| 4 | 2 | 3 | 0.561 | 0.546 | +0.015 |
| 6 | 2 | 2 | 0.571 | 0.539 | +..... |
| 7 | 1 | 2 | 0.571 | 0.550 | +..... |
| 8 | 1 | 2 | 0.580 | 0.547 | +..... |
| 9 | 1 | 2 | 0.571 | 0.548 | +..... |
| 10 | 1 | 1 | 0.572 | 0.542 | +..... |
| 11 | 1 | 1 | 0.562 | 0.543 | +0.019 |
| 12 | 1 | 1 | 0.572 | 0.551 | +..... |
| 13 | 1 | 1 | 0.540 | 0.542 | −0.002 |
| 14 | 1 | 1 | 0.549 | 0.538 | +0.011 |
| 15 | 1 | 3 | 0.561 | 0.538 | +0.023 |
| F 1 | 1 | 1 | 0.542 | 0.541 | +0.001 |
| 2 | 1 | 1 | 0.542 | 0.544 | −0.002 |
| 3 | 1 | 1 | 0.551 | 0.533 | +0.018 |
| 4 | 1 | 1 | 0.543 | 0.530 | +0.013 |
| 5 | 1 | 1 | 0.543 | 0.541 | +0.002 |
| 6 | 1 | 1 | 0.546 | 0.542 | +0.004 |
| 7 | 1 | 1 | 0.552 | 0.542 | +0.010 |
| 8 | 1 | 1 | 0.549 | 0.536 | +0.013 |
| 9 | 1 | 1 | 0.542 | 0.534 | +0.008 |
| 10 | 1 | 1 | 0.547 | 0.543 | +0.004 |
| 11 | 1 | 1 | 0.542 | 0.535 | +0.007 |
| J. H. | 1 | 1 | 0.560 | 0.546 | +0.014 |
| G. T. | 1 | 1 | 0.549 | 0.541 | +0.008 |
| A. D. | 1 | 1 | 0.550 | 0.548 | +0.002 |
| O. R. | 1 | 1 | 0.540 | 0.540 | ±0.000 |
| | *Herds* | | | | |
| Y | 1 | 1 | 0.553 | 0.540 | +0.013 |
| F | 1 | 4 | 0.560 | 0.539 | +0.021 |
| J. H. | 1 | 1 | 0.549 | 0.541 | +0.008 |
| G. T. | 1 | 1 | 0.553 | 0.553 | ±0.000 |
| A. D. | 1 | 1 | 0.540 | 0.540 | ±0.000 |
| O. R. | 1 | 1 | 0.533 | 0.533 | ±0.000 |

* Average.

TABLE 7.

*Variation in freezing-point depressions observed in milk from the same individual cow or the same herd on different days.*

| HERD AND COW NO. | NO. OF SAMPLES | | FREEZING-POINT VARIATION | | EXTREME RANGE |
|---|---|---|---|---|---|
| | A. M. | P. M. | A. M. | P. M. | |
| | *Individual Cows* | | °C. | °C. | °C. |
| Y 1 | 5 | 2 | 0.007 | 0.006 | 0.029 |
| 2 | 3 | 2 | 0.018 | 0.005 | 0.018 |
| 3 | 2 | 2 | 0.006 | 0.003 | 0.016 |
| 4 | 2 | 3 | 0.002 | 0.008 | 0.019 |
| 6 | 2 | 2 | 0.000 | 0.011 | ..... |
| 7 | 1 | 2 | ..... | 0.007 | ..... |
| 8 | 1 | 2 | ..... | 0.011 | ..... |
| 9 | 1 | 2 | ..... | 0.008 | ..... |
| 15 | 1 | 3 | ..... | 0.013 | 0 029 |
| | *Herd.* | | | | |
| F | 1 | 4 | ..... | 0.005 | 0.025 |

TABLE 8.

*Range in freezing-point depressions for normal milk.*

| | SPECIFIC GRAVITY | FAT | SOLIDS NOT FAT | FREEZING-POINT DEPRESSION |
|---|---|---|---|---|
| *Individual Cows.* | | | | |
| *Minnesota Dairy and Food Department—1919–1920 (60 samples).* | | | | |
| | | *per cent* | *per cent* | *–0°C.* |
| Maximum | 1.0350 | 7.30 | 10.15 | 0.562 |
| Minimum | 1.0262 | 2.20 | 7.37 | 0.534 |
| Average | 1.0319 | 3.94 | 8.90 | 0.547 |
| *—1921 (17 samples).* | | | | |
| Maximum | 1.0330 | 4.9 | 9.25 | 0.560 |
| Minimum | 1.0281 | 2.4 | 8.02 | 0.540 |
| Average | 1.0311 | 3.4 | 8.67 | 0.547 |
| *Libby, McNeill & Libby—1921 (27 samples).* | | | | |
| Maximum | 1.0380 | 6.3 | 10.26 | 0.563 |
| Minimum | 1.0256 | 0.9 | 7.20 | 0.532 |
| Average | 1.0313 | 3.9 | 8.62 | 0.546 |
| *Connecticut Agricultural Experiment Station—1921 (75 samples).* | | | | |
| Maximum | 1.0343 | 6.8 | 9.63 | 0.566* |
| Minimum | 1.0271 | 2.7 | 8.17 | 0.530* |
| Average | 1.0313 | 4.0 | 8.64 | 0.543* |
| *Herds.* | | | | |
| *Minnesota Dairy and Food Department—1919-1920 (15 samples).* | | | | |
| Maximum | 1.0330 | 5.50 | 9.27 | 0.562 |
| Minimum | 1.0305 | 3.10 | 8.48 | 0.545 |
| Average | 1.0319 | 4.15 | 8.95 | 0.551 |
| *Libby, McNeill & Libby—1921 (37 samples).* | | | | |
| Maximum | 1.0336 | 5.4 | 9.30 | 0.553 |
| Minimum | 1.0285 | 2.7 | 7.98 | 0.530 |
| Average | 1.0312 | 3.7 | 8.45 | 0.542 |
| *Connecticut Agricultural Experiment Station—1921 (9 samples).* | | | | |
| Maximum | 1.0317 | 4.1 | 8.77 | 0.560 |
| Minimum | 1.0305 | 3.3 | 8.38 | 0.535 |
| Average | 1.0311 | 3.6 | 8.50 | 0.544 |
| *Combined results of all collaborators—Individual cows (179 samples).* | | | | |
| Maximum | 1.0380 | 7.3 | 10.26 | 0.566 |
| Minimum | 1.0256 | 0.9 | 7.20 | 0.530 |
| Average | 1.0315 | 3.8 | 8.71 | 0.545 |
| *Herds (61 samples).* | | | | |
| Maximum | 1.0336 | 5.5 | 9.30 | 0.562 |
| Minimum | 1.0285 | 2.7 | 7.98 | 0.530 |
| Average | 1.0313 | 3.8 | 8.58 | 0.544 |

*Abnormal figures discussed elsewhere not included.

The chief purpose of the studies described in this report is to compare the results obtained by the referee last year with the data obtained from the wider observations of the several collaborators this year. The combined experience for the two years is shown in condensed form in Table 8.

From this condensed summary it appears that the tentative limits suggested last year are not substantially changed by the further observations made in collaborative study this year. For individual cows the new high of −0.530°C. and low of −0.566°C. are recorded as compared with −0.534°C. and −0.562°C. reported a year ago. For herd milk the new high figure of −0.530°C. was found but no new low figure was established. The average for individual cows as reported last year (−0.547°C.) remains practically unchanged (−0.545°C.) while the average for herd milk is raised from −0.551° to −0.544°C. The minimum depression observed for normal individual cows is −0.530°C., and the same figure obtains also for herd milk.

As shown in Table 3, long-delayed milking may or may not influence the freezing-point depression. In one of three cases where milkings were delayed from 9½ to 10½ hours, a conspicuous increase in depression occurred, but in the other two the changes were very slight, one of them being a decreased depression. No evidence is apparent that milking delayed from 1 to 3 hours produced any effect upon the freezing point.

Moderate exercise, such as a walk of four blocks, was without effect. In cases of severe exercise, strain or fatigue, such as walks of 5 to 6 miles, the freezing point was very materially lowered both for individuals and herds, the variations from normal being from 0.015 to 0.026°C. The comparisons between the normal and abnormal are true since the observations were made on the evening milk in both cases. In point of magnitude, certain of the abnormal figures (e. g. −0.571° and −0.578°C.) are at first glance suggestively coincident with the extremely low depressions observed in a number of morning samples discussed previously in this report, but they offer no valid explanation of them, because a delay of 10½ hours in milking does not occur in ordinary practice and because morning milk is drawn after a period of rest.

The observation that cows, to all outward appearances sound and healthy, may prove to be tubercular as judged by the tuberculin test, is not uncommon. This suggested the desirability of studying the freezing-point depressions of milk obtained from tubercular reactors and animals otherwise physically abnormal. Over 80% of the results reported by collaborators (Table 4) were within the limits observed for normal individuals and herds, but in 5 cases out of 32 unusually high freezing points (−0.520° to −0.523°C.) were obtained. The evidence in 3 of these 5 cases is, however, somewhat contradictory for the reason that samples drawn from the same cows after an interval of 2 months gave freezing points within the limits for normal milk. On the basis of these data, therefore, it would appear that while in many cases tuberculosis and other pathological conditions may not necessarily be reflected in the freezing-point depression of the milk, a few exceptionally high freezing points have been observed and these should be borne in mind when deciding the significance of depressions less than −0.530°C. in the case of milk from individual cows.

It was not possible to include in this report any adequate review of the literature dealing with the effect of pathological conditions upon the freezing points of secretions. Tieken[1] has shown comparisons between the freezing point of the blood and of various body fluids for a number of diseases in man. In the several cases of tuberculosis reported, the freezing-point of the blood remained normal (−0.56°C.), with the other fluids under observation closely corresponding. When conspicuous departures from normal were observed in the blood (e.g. in uremic coma) they were in the direction of *increased* depressions (−0.58 to −0.68°C.). Marked increase in depression of freezing point of the blood has been observed by some investigators in carcinoma, excessive

[1] H. Gideon Wells. Chemical Pathology, 1914, 324.

amounts of protein-decomposition products being regarded as the cause, but this experience has been questioned by others who found no such increase[1]. Koestler[2] investigated the detection of milk altered by secretion disturbances and found that pathological disturbances increase the serum nitrogen, chlorine and sodium and decrease the lactose, potassium and phosphorus. It is stated that the altered milk showed normal lowering of freezing-point and that this determination is a valuable check in cases where the general analytical results indicate added water. Further examination of this and other literature on the subject must be reserved for future study.

## CONCLUSIONS.

The complete data for the past two years represent the examination of 291 samples, distributed as follows:

| | |
|---|---|
| Normal individual cows | 179 |
| Normal herds | 61 |
| Diseased or otherwise abnormal individual cows | 37 |
| Diseased or otherwise abnormal herds | 3 |
| Unclassified, requiring further corroboration | 11 |
| Total | 291 |

The results indicate—

(1) That there is an appreciable, and often a conspicuous, difference in freezing-point depression between morning and evening milk. This morning-evening variation is greater than that observed between morning samples or evening samples on successive days.

(2) That the minimum freezing-point depression of −0.530°C. and maximum of −0.566°C. for milk from normal individual cows and the minimum of −0.530°C. and maximum of −0.562°C. for the milk from normal herds is reasonably substantiated by the experience of all collaborators.

(3) That from the data here reported the results of moderate exercise or moderately delayed milkings are not reflected in the freezing-point depressions of the milk. Long-delayed milkings, 9½ to 10½ hours, may or may not be followed by depressions varying from normal. Severe exercise, strain or fatigue is followed by materially increased depressions.

(4) That the milk from tubercular cows or those otherwise in poor or abnormal physical condition has generally fallen within the limits for normal milk as regards freezing points. The few exceptions noted have been in the direction of decreased depressions.

(5) That extremely low freezing points observed in certain samples of morning milk suggest a fuller investigation of this point. The study also of pathological conditions upon the freezing point may well be continued. The effect of increased acidity upon the freezing-point depression with a view to corroborating or modifying the correction factor suggested by Kiester[3] should be studied

[1] H. Gideon Wells. Chemical Pathology, 1914, 461.
[2] *Mitt. Lebensm. Hyg.*, 11: 154.
[3] *J. Ind. Eng. Chem.*, 1917, 9: 862.

# REPORT ON THE DETERMINATION OF MOISTURE IN CHEESE.

By LLOYD C. MITCHELL (U. S. Food and Drug Inspection Station, Minneapolis, Minn.), *Associate Referee.*

The tentative method for the determination of moisture in cheese[1] was studied with a view to its adoption as an official method. It is essentially the same as the method suggested by G. E. Patrick at the meeting of the association in 1907.

Preliminary to the work on the tentative method itself, the various factors which may affect the moisture results were studied. As deduced from literature and experience these factors[2] are—

(1) Type of oven used,

(2) Time of drying,

(3) Temperature,

(4) Pressure,

(5) Size of drying dish used,

(6) The presence or absence of porous material, such as asbestos or sand,

(7) Weight of sample used,

(8) Number of samples dried,

(9) Position of the sample in the oven (with certain types of ovens), and

(10) Current of air.

One pound of American cheese (Twin Daisy), purchased in the open market, was used in all the experiments. The samples were prepared according to the official method and kept about 20 hours in a quart Mason jar tightly closed before the experiments were started. Each sample was an arbitrary weight, varying from 5 to 5.5 grams. The number of samples dried also varied with each experiment. The numbers describing the apparatus used were taken from E. & A. Catalog AA of Chemical and Metallurgical Laboratory Supplies, 1920 Edition. The temperature was read at 5-minute intervals for the electric oven and at 15-minute intervals for both the vacuum and water-jacketed ovens.

Experiment I.—*In a high vacuum with a slow current of dry air*

(1) Vacuum, E. & A. 4893.

(2) 3 hours; 1 hour. Total, 4 hours.

(3) 63 to 65°C.; 58 to 67°C.

---

[1] *Assoc. Official Agr. Chemists, Methods*, 1920, 234.

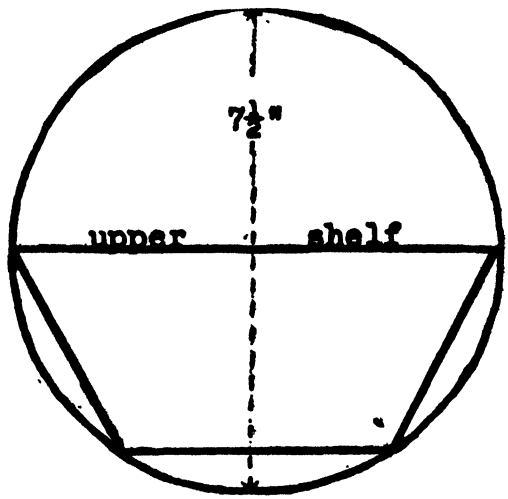
[2] Another factor, namely, the relative humidity within the oven during the entire drying period undoubtedly plays an important part in the moisture results. This factor was not studied because no suitable device was available to control the amount of humidity within the oven at the temperature at which the product was dried.

(4) Vacuum, 26 to 27 inches. (76-100 mm. of mercury) Bourdon vacuum gage, 3-inch size.

(5) Aluminum, flat bottom, with fit-over lid; diameter 90 mm., depth 15 mm., E. & A. 2604.

(6) None.

(9) Central portion on upper shelf.




CROSS SECTION OF VACUUM OVEN

(10) Obtained by bubbling air through sulfuric acid, specific gravity 1.84, at the rate of 2 bubbles per second.

TABLE 1.

*Moisture in cheese. (Experiment I.)*

| DISH 1* | DISH 2* | TEMPERATURE VARIATION | DRYING PERIOD |
|---|---|---|---|
| *per cent* | *per cent* | *°C.* | *hours* |
| 34.10 | 34.13 | 63–65 | 3 |
| 00.17 | 00.18 | 58–67 | 1 |
| 34.27 | 34.31 | 58–67 | 4 |

* Upper shelf—central

Experiment II.—*In electric oven at atmospheric pressure.*

(1) Freas electric, regular (Type R) No. 100, E. & A. 4816.

(2) One period 1 hour; 4 periods ½-hour each; 6 periods 1 hour each. Total, 9 hours.

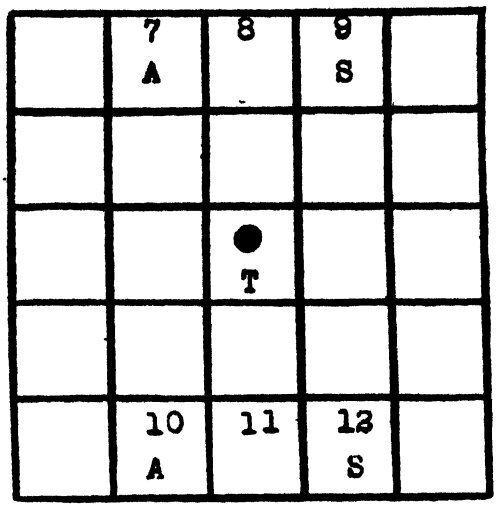
(3) 90 to 118°C. (See Table 2 for variations during different drying periods.)

(4) Atmospheric.

(5) Aluminum, flat bottom with slip-in lid; diameter 58 mm., depth 17 mm.; E. & A. 2605.

(6) Dishes, 1, 4, 7, and 10 contained approximately 1 gram each of cut asbestos; dishes 2, 5, 8, and 11, none; and dishes 3, 6, 9, and 12 contained approximately 5 grams each of sea-sand. All dishes were dried in the oven over night in the same relative positions as during the drying periods. Samples were placed on porous material, but not mixed with the material.

UPPER SHELF—BACK




Front

(9) Upper shelf, 6 inches above porcelain plate at bottom of oven.

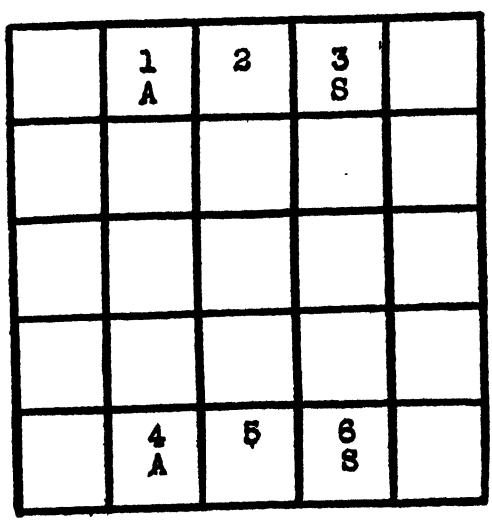
T—thermometer bulb 5 to 6 inches above porcelain plate and 6¼ inches from outer edge of shelf.

A—dishes containing asbestos.

S—dishes containing sand.

Numbers show relative position of the various dishes on shelf.

LOWER SHELF—BACK




Front

Lower shelf, 2 inches above porcelain plate at bottom of oven. A, S, and numbers, see description above for upper shelf.

TABLE 2.

*Moisture in cheese. (Experiment II.)*

| | | | | POROUS MATERIAL | | | | | | | | TEMPERATURE VARIATION | DRYING PERIOD |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | ASBESTOS | | | | SEA-SAND | | | | | |
| DISH 2* | DISH 5† | DISH 8‡ | DISH 11¶ | DISH 1* | DISH 4† | DISH 7‡ | DISH 10¶ | DISH 3* | DISH 6† | DISH 9‡ | DISH 12¶ | | |
| *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *°C.* | *hours* |
| 25.10 | 15.88 | 17.14 | 11.49 | 26.93 | 22.35 | 22.49 | 21.45 | 28.79 | 18.69 | 22.24 | 17.52 | 90-109 | 1 |
| 3.70 | 4.93 | 3.93 | 4.07 | 3.76 | 4.62 | 4.39 | 4.68 | 2.98 | 4.57 | 4.16 | 4.13 | 97-114 | ½ |
| 1.97 | 3.58 | 3.33 | 3.80 | 1.96 | 2.73 | 2.75 | 3.05 | 1.32 | 3.70 | 2.76 | 3.83 | 99-109 | ½ |
| 0.49 | 2.00 | 3.41 | 3.14 | 0.59 | 1.11 | 1.63 | 1.36 | 0.42 | 1.39 | 1.56 | 1.96 | 96-113 | ½ |
| 0.68 | 1.31 | 0.91 | 1.36 | 0.51 | 0.73 | 0.69 | 0.65 | 0.43 | 0.92 | 0.68 | 0.92 | 95-112 | ½ |
| 1.14 | 2.22 | 1.61 | 2.63 | 0.63 | 0.98 | 1.02 | 0.97 | 0.62 | 1.41 | 1.11 | 1.79 | 87-114 | 1 |
| 0.61 | 1.74 | 1.26 | 2.70 | 0.41 | 0.88 | 0.73 | 0.91 | 0.38 | 1.29 | 0.78 | 1.56 | 100-118 | 1 |
| 0.35 | 0.75 | 0.53 | 1.10 | 0.18 | 0.35 | 0.29 | 0.35 | 0.21 | 0.57 | 0.39 | 0.62 | 103-116 | 1 |
| 0.23 | 0.46 | 0.50 | 0.70 | 0.13 | 0.23 | 0.20 | 0.27 | 0.16 | 0.40 | 0.28 | 0.43 | 103-117 | 1 |
| 0.18 | 0.50 | 0.36 | 0.73 | 0.16 | 0.26 | 0.19 | 0.25 | 0.09 | 0.38 | 0.19 | 0.37 | 106-117 | 1 |
| 0.14 | 0.21 | 0.24 | 0.36 | 0.12 | 0.13 | 0.10 | 0.14 | 0.07 | 0.21 | 0.15 | 0.23 | 103-118 | 1 |
| 34.59 | 33.58 | 33.22 | 32.08 | 35.38 | 34.37 | 34.48 | 34.08 | 35.47 | 33.53 | 34.30 | 33.36 | 90-118 | 9 |

* Lower shelf—back.
† Lower shelf—front.
‡ Upper shelf—back.
¶ Upper shelf—front.

Experiment III.—*In a high vacuum with a slow current of dry air.*

(1) See Experiment I (1).
(2) 9 periods, 2 hours each. Total, 18 hours.
(3) 58 to 78°C. (See Table 3 for variations during various drying periods.)
(4) See Experiment I (4).
(5) See Experiment II (5).
(6) Dishes 1, 4, and 7 contained approximately 1 gram each of cut asbestos; dishes 2, 5, and 8, none; and dishes 3, 6, and 9 contained approximately 5 grams each of sea-sand. All dishes were dried over night in oven. Samples were placed on porous material, but not mixed with the material.
(10) See Experiment I (10).

TABLE 3.

*Moisture in cheese. (Experiment III.)*

| | | | POROUS MATERIAL | | | | | | TEMPERATURE VARIATION | DRYING PERIOD |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | ASBESTOS | | | SEA-SAND | | | | |
| DISH 2* | DISH 5† | DISH 8‡ | DISH 1* | DISH 4† | DISH 7‡ | DISH 3* | DISH 6† | DISH 9‡ | | |
| *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *°C.* | *hours* |
| 21.88 | 22.27 | 23.44 | 26.55 | 27.69 | 25.90 | 25.16 | 26.13 | 25.83 | 60–67 | 2 |
| 3.31 | 2.82 | 2.34 | 4.23 | 3.74 | 4.68 | 3.79 | 3.44 | 3.45 | 59–64 | 2 |
| 2.89 | 2.62 | 2.48 | 1.96 | 1.70 | 2.08 | 2.26 | 2.02 | 2.12 | 63–68 | 2 |
| 1.55 | 1.37 | 1.26 | 0.65 | 0.58 | 0.67 | 0.90 | 0.81 | 0.83 | 60–69 | 2 |
| 1.35 | 1.30 | 1.26 | 0.48 | 0.43 | 0.51 | 0.74 | 0.65 | 0.74 | 64–72 | 2 |
| 0.82 | 0.92 | 0.84 | 0.33 | 0.29 | 0.34 | 0.46 | 0.45 | 0.42 | 67–75 | 2 |
| 0.93 | 1.10 | 0.96 | 0.21 | 0.16 | 0.15 | 0.45 | 0.38 | 0.41 | 67–77 | 2 |
| 0.38 | 0.43 | 0.33 | 0.27 | 0.36 | 0.29 | 0.29 | 0.27 | 0.27 | 68–74 | 2 |
| 0.32 | 0.37 | 0.30 | 0.17 | 0.16 | 0.16 | 0.21 | 0.20 | 0.19 | 72–78 | 2 |
| 33.43 | 33.20 | 33.21 | 34.85 | 35.11 | 34.78 | 34.26 | 34.35 | 34.26 | 59–78 | 18 |

* Upper shelf—back.
† Upper shelf—central.
‡ Upper shelf—front.

Experiment IV.—*In water-jacketed oven.*

(1) Hot water, double wall, E. & A. 4876; size, outside, 10x10x12 inches.
(2) 3 hours; 1½ hours. Total, 4½ hours.
(3) 93 to 96°C.
(4) Atmospheric.
(5) Platinum, flat bottom, straight sides; diameter 85 mm., depth 20 mm.; with glass rod about 5 mm. in diameter and slightly longer than diameter of dish.
(6) Dishes 1 and 4 contained approximately 2 grams each of cut asbestos; dishes 2 and 5, none; and dishes 3 and 6 contained approximately 15 grams each of sea-sand. All dishes were ignited at dull red heat in an electric muffle. Samples were thoroughly mixed with the porous materials.

TABLE 4.
*Moisture in cheese. (Experiment IV.)*

| | | POROUS MATERIAL | | | | TEMPERATURE VARIATIONS | DRYING PERIOD |
|---|---|---|---|---|---|---|---|
| | | ASBESTOS | | SEA-SAND | | | |
| DISH 2* | DISH 5† | DISH 1* | DISH 4† | DISH 3* | DISH 6† | | |
| *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *°C.* | *hours* |
| 32.01 | 29.97 | 34.76 | 34.42 | 34.92 | 34.70 | 93–96 | 3 |
| 1.59 | 2.35 | 0.32 | 0.26 | 0.30 | 0.16 | 97–98 | 1½ |
| 33.60 | 32.32 | 35.08 | 34.68 | 35.22 | 34.86 | 93–98 | 4½ |

* Bottom of oven—central.
† Upper shelf—central.

Experiment V.—*In a high vacuum with a slow current of dry air.*

(1) See Experiment I (1).
(2) 1 period 3 hours; 3 periods 1½ hours each. Total, 7½ hours.
(3) 89 to 98.5°C. (See Table 5 for variations during different drying periods.)
(4) See Experiment I (4).
(5) See Experiment IV (5).
(6) Dishes 1, 3, and 5 contained approximately 2 grams each of finely divided ignited asbestos; and dishes 2, 4, and 6 contained approximately 15 grams each of ignited sea-sand. Samples were thoroughly mixed with porous material.
(10) See Experiment I (10).

TABLE 5.
*Moisture in cheese. (Experiment V.)*

| POROUS MATERIAL | | | | | | TEMPERATURE VARIATIONS | DRYING PERIOD |
|---|---|---|---|---|---|---|---|
| ASBESTOS | | | SEA-SAND | | | | |
| DISH 1* | DISH 3† | DISH 5‡ | DISH 2* | DISH 4† | DISH 6‡ | | |
| *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *°C.* | *hours* |
| 33.11 | 33.23 | 33.06 | 33.64 | 33.58 | 34.24 | 89-98.5 | 3 |
| 0.20 | 0.21 | 0.16 | 0.15 | 0.19 | 0.27 | 96-98.5 | 1½ |
| 0.13 | 0.14 | 0.48 | 0.24 | 0.18 | 0.31 | 97-98.5 | 1½ |
| 0.12 | 0.31 | 0.10 | 0.12 | 0.19 | 0.50 | 97-98.5 | 1½ |
| 33.56 | 33.89 | 33.80 | 34.15 | 34.14 | 35.32 | 89-98.5 | 7½ |

* Upper shelf—back.
† Upper shelf—central.
‡ Upper shelf—front.

Experiment VI.—*In a high vacuum with a slow current of dry air.*

(1) See Experiment I (1).

(2) 1 period 3 hours; 3 periods 1½ hours (low temperature); 1 period 2 hours (high temperature). Total, 9½ hours.

(3) 57–72°C., then 95–98°C. (See Table 6 for variations during different drying periods.)

(4) See Experiment I (4).

(5) See Experiment IV (5).

(6) Dishes 1 and 4 contained none; dishes 2 and 5 contained approximately 2 grams each of finely divided, ignited asbestos; and dishes 3 and 6 contained approximately 15 grams each of ignited sea-sand. Samples were thoroughly mixed with the porous material.

(10) See Experiment I (10).

TABLE 6.

*Moisture in cheese. (Experiment VI.*)*

| | | POROUS MATERIAL | | | | TEMPERATURE VARIATIONS | DRYING PERIOD |
|---|---|---|---|---|---|---|---|
| | | ASBESTOS | | SEA-SAND | | | |
| DISH 1 | DISH 4 | DISH 2 | DISH 5 | DISH 3 | DISH 6 | | |
| *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *°C* | *hours* |
| 28.43 | 26.60 | 31.14 | 31.44 | 31.91 | 31.35 | 57-66 | 3 |
| 1.87 | 3.72 | 0.60 | 0.43 | 0.45 | 0.39 | 62-72 | 1½ |
| 0.51 | 0.57 | 0.33 | 0.47 | 0.21 | 0.52 | 59-65 | 1½ |
| 0.40 | 0.41 | 0.28 | 0.25 | 0.31 | 0.18 | 63-68 | 1½ |
| 0.30 | 0.31 | 0.05 | 0.10 | 0.07† | 0.14 | 60-68 | 1½ |
| 1.55 | 1.17 | 0.72 | 0.84 | 0.60 | 0.74 | 95-98 | 2 |
| | | | | | | 57-72 | 9 |
| 33.06 | 32.78 | 33.12 | 33.53 | 33.41 | 33.32 | 95-98 | 2 |

* All dishes were placed on upper shelf—central.
† Increase.

## DISCUSSION.

The experiments show that the electric oven used in Experiment II was unsatisfactory. The temperature was read every 5 minutes and showed in many instances a variation as high as 10 to 15°C. Take the eleventh drying period of one hour for example. The temperature immediately after the 12 samples were placed into the oven and the door was closed was 96°C.; then at successive 5-minute intervals it showed 118, 115, 107, 106, 114, 108, 105, 116, 108, 103, 117, and 111°C. with no attempt at regulation. This wide variation in temperature within the oven is more strikingly brought out in a study of the results showing the loss of moisture during the first drying period of one hour as reported in Table 2. The thermometer immediately after the 12 samples were placed in the oven and the door was closed was 90°C.; then at 5-minute intervals it was 98, 90, 96, 102, 105, 100, 103, 104, 105, 102, 103, and 103°C. Dishes 2, 5, 8, and 11, identical in every respect

TABLE 7.

*Summary of moisture results in cheese.*

| EXPERIMENT | OVEN | TOTAL TIME | TEMPERATURE VARIATION | PRESSURE | SIZE OF DISH | POSITION OF DISH IN OVEN | TOTAL MOISTURE | LAST DRYING PERIOD | | POROUS MATERIAL | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | ASBESTOS | | | SEA-SAND | | |
| | | | | | | | | | | Total Moisture | Last Drying Period | | Total Moisture | Last Drying Period | |
| | | | | | | | | Moisture | Time | | Moisture | Time | | Moisture | Time |
| | | *hours* | *°C.* | *inches* | *mm.* | | *per cent* | *per cent* | *hours* | *per cent* | *per cent* | *hours* | *per cent* | *per cent* | *hours* |
| I | Vacuum | 4 | 58–67 | 26–27 | 90x15 | Upper shelf—middle | 34.27<br>34.31 | 0.17<br>0.18 | 1 | | | | | | |
| II | Electric | 9 | 90–118 | Atmospheric | 58x17 | Lower shelf—back<br>Lower shelf—front<br>Upper shelf—back<br>Upper shelf—front | 34.59<br>33.58<br>33.22<br>32.08 | 0.14<br>0.21<br>0.24<br>0.36 | 1 | 35.38<br>34.37<br>34.48<br>34.08 | 0.12<br>0.13<br>0.10<br>0.14 | 1 | 35.47<br>33.53<br>34.30<br>33.36 | 0.07<br>0.21<br>0.15<br>0.23 | 1 |
| III | Vacuum | 18 | 59–78 | 26–27 | 58x17 | Upper shelf—back<br>Upper shelf—middle<br>Upper shelf—front | 33.43<br>33.20<br>33.21 | 0.32<br>0.37<br>0.30 | 2 | 34.85<br>35.11<br>34.78 | 0.17<br>0.16<br>0.16 | 2 | 34.26<br>34.35<br>34.26 | 0.21<br>0.20<br>0.19 | 2 |
| IV | Water | 4½ | 93-96 | Atmospheric | 85x20 | Bottom of oven—middle<br>Upper shelf—middle | 33.60<br>32.32 | 1.59<br>2.35 | 1½ | 35.08<br>34.68 | 0.32<br>0.26 | 1½ | 35.22<br>34.86 | 0.30<br>0.16 | 1½ |
| V | Vacuum | 7½ | 89-98.5 | 26–27 | 85x20 | Upper shelf—back<br>Upper shelf—middle<br>Upper shelf—front | | | | 33.56<br>33.89<br>33.80 | 0.12<br>0.31<br>0.10 | 1½ | 34.15<br>34.14<br>35.32 | 0.12<br>0.19<br>0.50 | 1½ |
| VI | Vacuum | 9 | 57–72 | 26–27 | 85x20 | Upper shelf | 33.06 | 0.30*<br>1.55† | 1½*<br>2† | 33.12 | 0.05*<br>0.72† | 1½*<br>2† | 33.41 | 0.07*‡<br>0.60† | 1½*<br>2† |
| | | 2 | 95–98 | | | Central portion | 32.78 | 0.31*<br>1.17† | 1½*<br>2† | 33.53 | 0.10*<br>0.84† | 1½*<br>2† | 33.32 | 0.14*<br>0.74† | 1½*<br>2† |

* Loss of moisture during and the time of last drying period at low temperature.
† Loss of moisture during and the time of last drying period at high temperature.
‡ Increase.

except location within the oven, showed a variation of over 14 per cent; dishes 1, 4, 7, and 10, over 5 per cent; and dishes 3, 6, 9, and 12, over 11 per cent. Both the variations in temperature and the differences in moisture results indicate that no two places in the oven were heated alike and that the temperature was fluctuating continuously over a wide range. A similar condition showing the wide variation of temperature within an electric oven was reported in June, 1921, from the Bureau of Chemistry, where it was observed that with the thermometer in the center of the oven (Freas electric) registering 100°C., a thermometer lying on the porcelain plate at the bottom reached a temperature of 160°C. It is the opinion of your referee that no oven heated with the heating units within the drying chamber in the manner in which the Freas electric oven is constructed, without some more suitable means of circulation, will prove satisfactory for moisture determinations where known and constant temperatures are required.

The results found in Experiments I, III, V, and VI indicate that the temperature was practically uniform throughout the drying chamber of the vacuum oven, and that a similar statement may be made from Experiment IV regarding the water-jacketed oven.

The time required to dry the cheese to the point where the loss for a given drying period is 0.2 per cent. or lower, apparently varies with every change of condition. In Experiment I this point was reached within 4 hours, while in Experiment III, where no porous material was used, it was not reached within 18 hours. It was just barely reached where sand or asbestos was employed. The only difference between these two experiments, when the porous material was omitted, was in the size of dishes used and the number of samples dried. It was noted, however, that in Experiment I the fat in the cheese did not melt and run over the bottom of the dishes, whereas it did in Experiment III. In Experiment VI, using practically the same sized dish but drying a smaller number of samples than in Experiment III, the loss within two-tenths of one per cent in a given drying period was reached within 9 hours for the samples when porous materials were employed, and slightly above this amount when they were omitted. The samples lost from 0.60 to 1.55 per cent more in weight when they were dried for 2 hours longer, raising the temperature from 57-72°C. to the boiling point of water. This would seem to show that while the samples were approaching a constant weight at the lower temperature, a greater loss in weight would be obtained at a higher temperature. In Experiment V, similar to Experiment VI, except that porous materials were employed in all 6 dishes instead of 4 with and 2 without and that the temperature was raised to the boiling point of water instead of around 60 to 70°C., the loss within the two-tenths of one per cent was reached within 4½ hours. With further drying the results became erratic, some above and

some below the two-tenths of one per cent loss. In Experiment IV, when the water-jacketed oven was used at atmospheric pressure, the loss in weight was between two- and three-tenths of one per cent within 4½ hours for those samples where the porous materials were employed, but from 1.59 to 2.35 per cent where they were omitted. In Experiment II, using the electric oven, the time required for the various dishes to reach the 0.2 per cent loss varied with the location of the dishes within the oven.

At the boiling point of water, satisfactory results, apparently, are obtained by drying either at atmospheric pressure or in a high vacuum. At a lower temperature, i. e. around 70°C., and with a high vacuum, Experiment VI seems to indicate that the moisture results would be too low.

It will be further noted that the moisture results are invariably lower, not only for the initial drying period but also for the final loss, when the porous material was omitted, than when either sand or asbestos was used. Your referee prefers to employ sea-sand rather than asbestos. Sea-sand seems easier to manipulate, while asbestos shows a slight tendency to absorb moisture when weighing.

## CONCLUSIONS.

It may be concluded that cheese may be dried most favorably and within the shortest time by the use of a large dish, when the sample is intimately mixed with either sand or asbestos and kept at the temperature of the boiling point of water at atmospheric pressure or in a high vacuum. Some analysts may prefer to use 10-15 grams of sea-sand rather than 2-3 grams of asbestos as given in the method, and to dry at the temperature of boiling water in a high vacuum rather than at atmospheric pressure.

## RECOMMENDATIONS.

It is recommended—

(1) That the tentative method be rewritten so as to include (a) that either 10-15 grams of sea-sand or 2-3 grams of asbestos be used, and (b) that the sample be dried either in a vacuum or at atmospheric pressure at the boiling point of water.

(2) That the terms "in a vacuum" or "in vacuo" be specifically defined. It is suggested that 26-27 inches (75-100 mm. mercury) be used.

(3) That the present tentative method with the changes recommended be further studied with a view to its adoption as an official method.

# DETERMINATION OF FAT IN MALTED MILK.

By J. T. KEISTER (Bureau of Chemistry, Washington, D. C.), *Associate Referee.*

Owing to the lateness in starting this work, the advance in the date of the meeting and delay in obtaining the samples, few results were obtained.

As the opinion had been expressed that one difficulty in determining accurately the fat in malted milk is due to the lack of uniformity or unequal distribution of the fat, it was thought desirable to determine this point, if possible, by noting any differences in results obtained upon samples made by different methods. Accordingly, one of the manufacturers of malted milk furnished two representative samples made by two different processes, known as the "drum" and "pan" processes.

Two sets only of these samples were sent to collaborators. Results on one set were received, but the other collaborator could not report in time for the meeting.

Two samples representing two different makes of malted milk had been collected previously on the market and sub-divisions had been submitted to three collaborators outside of Washington and to members of the Food Control Laboratory, with instructions to make fat determinations by the official Roese-Gottlieb method[1] and also by the neutral procedure; i. e., by omitting the use of ammonia in the official method. The few results returned agree with those obtained by the writer last year, in that the neutral process extracts the fat more completely. The difference is usually 0.05 to 0.2 per cent.

It will be noted that a small amount of fat is obtained by a third extraction when ammonia is used, which rarely occurs otherwise. This indicates that the ammonia does not facilitate, but rather makes the complete extraction of fat more difficult.

It is believed that the neutral procedure is a decided improvement over the regular official method as applied to malted milk, and that the wide variation in results obtained in the past is due principally to the treatment of the sample previous to extraction and to small errors introduced in treating a small sample.

A few experiments were made with one brand of malted milk in which a modification was introduced during the preparation of the sample. The results indicate that greater uniformity can be obtained. This modification will be applied to all the different brands, and the results will be tabulated and reported to the referee before the next meeting of the association.

[1] *Assoc. Official Agr. Chemists, Methods*, 1920, 227.

*Determination of fat in malted milk.*

(All results calculated to water-free basis.)

| ANALYST | SAMPLE | NEUTRAL METHOD | ROESE-GOTTLIEB METHOD |
|---|---|---|---|
| | | *per cent* | *per cent* |
| D. B. Scott, Bureau of Chemistry, Washington, D. C. | A | 9.19<br>9.26 | 9.04<br>8.93 |
| J. T. Keister | A | 8.93<br>9.05<br>8.99 | 8.59<br>8.84 |
| D. B. Scott | B | 9.28<br>9.26 | 9.09<br>9.04 |
| J. T. Keister | B | 9.14<br>9.07<br>9.14 | 9.06<br>9.09<br>9.07 |
| L. W. Ferris, Bureau of Chemistry, Washington, D. C. | C | 9.35<br>9.46<br>9.47 | 9.13<br>9.16 |
| Mr. Nason, Borden Condensed Milk Co., New York, N. Y. | C | 9.59<br>9.72 | 9.32<br>9.35 |
| J. T. Keister | C | 9.45<br>9.49<br>9.64 | 9.15<br>9.33<br>9.27 |
| L. W. Ferris | D | 9.24<br>9.17 | 9.27<br>9.23 |
| Mr. Nason | A | 9.35<br>9.24 | 9.14<br>9.03 |
| J. T. Keister | B | 9.45<br>9.25<br>9.31 | 9.01<br>9.05<br>9.27 |

It is recommended that a study of the neutral method for fat in malted milk be continued for another year with the view of recommending to the association some method for adoption.

This method is as follows:

Weigh accurately about 1 gram of the well-mixed sample into a small-lipped beaker, add 3 cc. of warm distilled water, stir with a glass rod until all lumps disappear and transfer to a Röhrig tube or similar apparatus. Rinse out beaker with 7 cc. more of water and transfer to the tube. Add 10 cc. of 95% alcohol and shake; then add 25 cc.

of ethyl ether and shake for at least 1 minute and follow with 25 cc. of petroleum ether, boiling point below 65°C., and shake thoroughly. Let settle until clear and draw off through a small filter paper into a weighed flask. Repeat the extraction, using the same amount of ethers and draw off into the same flask. Distil or evaporate off the ethers and dry in the oven at 100°C. to constant weight (1-hour intervals). Make a third extraction using 15 cc. of each ether, drawing off into a separate flask. In case an emulsion formation is noticed, add a few additional cc. of alcohol in the third extraction. (Extraction is usually complete after two extractions.)

After presenting his report, "Determination of Fat in Malted Milk", Keister suggested that the following change be made in the official method relating to condensed milk (unsweetened)[1]: Change the sentence, "Dilute 40 grams of the homogenous sample with 60 grams of water and proceed as directed under **1** to **15**, inclusive", to read as follows: "Dilute 40 grams of the homogenous sample with 60 grams of water and proceed as directed under **1** to **12**, inclusive". He explained that No. 13 of these methods (the regular Babcock method for fat in fresh milk) was not applicable to a condensed product.

## THE MOISTURE CONTENT OF DRIED MILK.

By George E. Holm (Dairy Division, Bureau of Animal Industry, Washington, D. C.).

In 1917[2], the Referee on Dairy Products, recommending further study of methods for the determination of moisture in milk products including dried milk, showed that the moisture content of samples of malted and dried milk varied from 2.88 to 7.65 per cent, and that constant weight could not be obtained by drying upon a water bath, but that two hours' heating in a vacuum oven at 95°C. is necessary for complete drying of a sample.

In this paper the writer will endeavor to point out some of the difficulties encountered in handling various milk powders and to show that consistent results can be obtained only when samples are carefully guarded against available moisture.

When samples of milk powder, made by a spray or vacuum process in which the heat was not sufficiently high to destroy the colloidal properties, were placed in desiccators over concentrated sulfuric acid at 25°C. it was found that after 7 to 10 days the moisture content of the samples became approximately constant, but that they still contained more than 1 per cent of moisture. This shows the great avidity that these powders have for moisture. When, however, the powder had been subjected to high temperature during its manufacture, its adsorption

[1] *Assoc. Official Agr. Chemists, Methods*, 1920, 231.
[2] *J. Assoc. Official Agr. Chemists*, 1920, 4: 201.

properties were lost to a certain extent, and it could be dried completely or nearly so over concentrated sulfuric acid.

The following table shows a comparison of the moisture content of these two types of milk powders, dried to approximately constant weight over concentrated sulfuric acid.

TABLE 1.

*Comparison of moisture content of two types of milk powders.*

| SAMPLE NO. | TOTAL MOISTURE CONTENT | MOISTURE CONTENT AT CONSTANT WEIGHT OVER CONCENTRATED SULFURIC ACID |
|---|---|---|
| | *per cent* | *per cent* |
| 1 | 2.39 | 1.54 |
| 2 | 2.61 | 1.21 |
| 3* | 2.34 | 0.22 |
| 4* | 2.18 | 0.00 |

* Relatively severe heat treatment was given during manufacture.

It had also been noted repeatedly that when a powder was exposed to the atmosphere the moisture content would increase rapidly. With these factors in mind, the following procedure was followed in the determination of moisture on dried-milk samples:

The milk powder was placed in a tightly sealed Mason jar and exposed to the air only when necessary to remove each sample. Weighing bottles with ground glass covers were used in all determinations. A sample of powdered milk (approximately 2 grams) was transferred from the Mason jar to the weighing bottle, the cover replaced to insure against adsorption of moisture from the atmosphere, and the sample weighed. The cover was removed and the sample placed in a water-jacketed vacuum oven, operating under partial vacuum (approximately 25 inches). A drying bottle containing sulfuric acid was attached to the drying chamber through which air was admitted very slowly, thus permitting a more complete removal of the vapors in the oven. The sample was kept under the desired conditions until removed and the cover replaced. After cooling in a desiccator over sulfuric acid for 15 to 20 minutes the sample was weighed.

TABLE 2.

*Moisture content of samples of milk made by the spray process.*

| DRYING TEMPERATURE | DRYING PERIOD | | | | | REMARKS |
|---|---|---|---|---|---|---|
| | ½ hour | 1 hour | 2 hours | 3 hours | 4 hours | |
| *cc.* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | |
| 50 | | | 3.04 | | | Low |
| 80 | | 4.02 | 3.94 | 4.02 | | Constant but not complete. |
| 90 | | 4.34 | | | | Almost complete. |
| 100 | | 4.43 | 4.38 | | 4.38 | Complete after 1 hour. |
| 100 | 4.25 | 4.38 | 4.36 | | | Complete after 1 hour. |

The results given in Table 2 were obtained with a sample of milk powder made by the spray process, using varying temperatures and lengths of time in drying.

At 50°C. the drying was exceedingly slow and incomplete; even at 80°C. the sample did not dry completely nor progressively with increased time of drying. At 90°C. the drying was almost complete in one hour, but there was no advantage over drying at 100°C., provided this temperature could be used.

It is inadvisable to use temperatures above 100°C. since it has been reported by N. Schoorl and S. C. L. Gerritzen[1] that lactose decomposes at 103°C. in the presence of phosphates.

Several experiments at 100°C. proved that drying in partial vacuum for one hour at this temperature was sufficient. As seen in Table 2, further continued drying causes no loss of weight. Determinations made by another analyst, following the same directions, resulted in a moisture content of 4.38 per cent on the same sample, which is in perfect agreement with the results shown in this table.

Shipping in paper containers is by no means an absolute safeguard against changes in the moisture content of samples. Milk powder of relatively high moisture content which has been sealed in paper cartons and placed in an atmosphere of relatively high humidity (60 per cent) will absorb moisture rapidly. Samples of low moisture content under the same conditions adsorb moisture from atmospheres of relatively low humidity.

## CONCLUSIONS.

The results show that accurate and concordant moisture determinations can be made on dried milk if precautions are taken to guard the samples from contact with moisture while they are being handled.

To insure absolutely reliable results in control methods or in any work upon this product, it is necessary to safeguard the products by shipping them in tightly sealed glass or metal containers, since changes in the moisture content mean heterogeneous and unreliable results with regard to other determinations, such as fats, proteins, etc.

[1] *Pharm. Weekblad*, 1921, 58: 370.

## REPORT ON FATS AND OILS.

By G. S. JAMIESON (Bureau of Chemistry, Washington, D. C.), *Referee.*

It is recommended—

(1) That final action be taken to make official the Wijs method[1] for the determination of iodine absorption number.

(2) That work be undertaken to obtain experimental data to prove that in the determination of the iodine absorption of an oil by the official Hanus procedure, it would be preferable to allow the Hanus solution to act on the oil for 45 minutes instead of 30 minutes, as stated in the official method. For some years it has been the practice in the Oil, Fat and Wax Laboratory, Bureau of Chemistry, to allow the Hanus solution to react on the oil for 45 minutes with the belief that more uniform and better results were obtained.

(3) That after suitable investigation a more comprehensive description be prepared in connection with the test for the detection of sesame oil in the presence of olive oil in order to guide the chemist when testing olive oils, such as those of Spanish or African origin, which frequently give pink or even crimson colorations with the official tests. Since the liquid fatty acids of these oils do not give pink or crimson color when the official tests are applied, it is recommended that the testing of these acids be added to the official methods. These important changes should be incorporated into the present methods as soon as possible as very large amounts of Spanish olive oil are being imported into this country.

## MODIFIED PROCEDURE FOR THE DETERMINATION OF THE "TURBIDITY POINT" OF BUTTER FAT.

By ARMIN SEIDENBERG (Chemical Laboratory, Department of Health, New York, N. Y.).

The following procedure for the determination of the "turbidity point" is a modification, in detail only, of that described in the original paper[2]. While it is more accurate and convenient, it does not affect the constants that have been established. Besides the particular "turbidity point" described here, it is possible to determine on the same sample any number of other "turbidity points" that may be desired by varying any one or more of a number of factors such as temperature, amount of dehydrated alcohol (or of 90 or 95 per cent alcohol), etc.

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[1] *Assoc. Official Agr. Chemists, Methods*, 1920, 245.
[2] *J. Ind. Eng. Chem.*, 1918, **10**: 617.

Weigh 10 grams of the well-mixed sample into a beaker, dissolve in an ether-alcohol solution made up by adding from a double graduated pipet 10 cc. of dehydrated alcohol (1) to 90 cc. of ethyl ether and shake well. Use several portions of this mixture to transfer the fat from the beaker into a 200 cc. graduated cylinder 30x3 cm. Dilute the solution to about 96 cc. so that when the tubing and thermometer are immersed it will reach the 100cc. mark on the cylinder. Through an accurately fitted rubber stopper pass a thermometer and two pieces of glass tubing with an outside dimension of approximately 0.5 cm. (One tube should reach to the bottom of the cylinder; the other to just below the stopper.) Use a thermometer (2) approximately 0.5 cm. in diameter and so graduated that each degree takes up a space of approximately 0.5 cm. It should reach to below the 45 cc. mark on the cylinder. Cut two circular pieces, 0.2—0.3 cm. in width, from a narrow bore black rubber tubing and pass over the thermometer, one just above the 13° mark and the other just below the 12° mark. Place the thermometer near the side of the cylinder.

Attach the cylinder to a ring stand with the bottom of the cylinder about 30 cm. above the base of the stand. Place a tall beaker containing water at 30–40°C. upon a ring loosely attached to the stand so that it can be raised or lowered readily, the weight of the beaker and water holding the ring in position. Aspirate air (3) through the solution in the cylinder at a rate (4) such that the volume is reduced from 100 to 60 cc. in about 10 minutes (not more than 12 and not less than 8 minutes). Raise the beaker when the temperature of the solution reaches 13° so that part of the cylinder is immersed in the water. Adjust the part of the cylinder immersed by raising or lowering the beaker to keep the temperature between 12 and 13°C. It is usually possible, after some trials, to adjust the beaker so as to keep the temperature constant. As a rule turbidity increases gradually. Take the "turbidity point" where a black object can not be seen through the solution in reflected light (5).

## COMMENTS.

(1) Alcohol must be measured accurately and should be 98-99 per cent by volume; it should contain about 1.5 per cent water by volume.

(2) If desired, a magnifying glass may be attached by a clamp to the stand holding the cylinder and arranged so that it bears on the part of the scale at which the temperature is to be maintained; the rubber bands will further facilitate in marking this part of the scale.

(3) Apparently no particular advantage was secured in first passing the air through alcohol.

(4) If the rate of suction is varied to any extent the result is affected to a considerable degree.

(5) In determining the exact point at which the solution may be considered to have become turbid the view should not be directly toward a light.

# REPORT ON BAKING POWDER.

By L. H. BAILEY (Bureau of Chemistry, Washington, D. C.), *Referee.*

The work for 1921, following the recommendations of Committee C for 1920, included a further study of the Chittick method[1] for the determination of lead in baking powder; a study of the details of the electrolytic method[2] (Corper-Bryan) for the determination of lead with special reference to acidity conditions during electrolysis; a study of the methods for the determination of the neutralizing strength of baking acids; a collaborative study of the determination of fluorine in baking powders and phosphates; and a study of the method for the determination of carbon dioxide in baking powder by a volumetric method[3] described by C. S. Robinson.

*Determination of lead by the Gravimetric method.*

The samples sent to the collaborators were prepared through the courtesy of J. R. Chittick, (Jaques Manufacturing Company, Chicago, Ill.). From analysis of constituents, the baking powder contained 3 parts per million of lead; to this was added 50 parts per million of lead in the form of sulfate, making the sample contain a total of 53 parts per million.

The Chittick method, having been recently modified by the author, was submitted for collaborative study. The method in its modified form follows:

MODIFIED CHITTICK METHOD.

PREPARATION OF REAGENTS.

*Sulfuric acid (1 to 5).*—Mix 500 cc. of 95% sulfuric acid, C. P., with 2500 cc. of water, let stand overnight, and filter.

*Acid-alcohol-water mixture.*—Mix 80 cc. of 95% sulfuric acid, C. P., with 3 liters of water. Then add 800 cc. of redistilled 95% alcohol (methyl or ethyl), stir thoroughly, let stand overnight, and filter.

*Glacial acetic acid.*—Redistil C. P. glacial acetic acid and store in bottles made from lead-free glass.

*Alkaline ammonium acetate solution.*—Mix 350 cc. of redistilled glacial acetic acid with 650 cc. of water; dilute 500 cc. of C. P. ammonium hydroxide (sp. gr. 0.90) with 500 cc. of water; then mix the two solutions. Store in bottles made from lead-free glass.

*Potassium chromate solution.*—Dissolve 65 grams of C. P. potassium chromate in 100 cc. of water, heating gently. Allow the solution to come to room temperature and filter.

DETERMINATION.

Weigh 100 grams of the thoroughly mixed sample and place in a 2 liter lipped beaker Add, in small portions, 750 cc. of dilute sulfuric acid (1 to 5). When frothing has

[1] *J. Assoc. Official Agr. Chemists*, 1920, **4**: 218.
[2] *Ibid.*, 221.
[3] *Ibid.*, 1921, **5**: 185; *Soil Science*, 1920, **10**: 41.

ceased, mark the volume of the mixture on the side of the beaker. Heat on the hot plate to boiling and continue boiling for 3–4 minutes; then heat on the steam bath until the starch is hydrolyzed, which requires 20–30 minutes. The mixture will have a yellow color. (See Note 1, below.)

Remove and add, while stirring, C. P. calcium sulfate which has been finely powdered in a mortar and rubbed with water to a thin paste.

> Monocalcium phosphate baking powder does not need the addition of calcium sulfate, since of itself it forms sufficient insoluble residue. To combination baking powders containing in part monocalcium phosphate, add 10 grams of calcium sulfate. To all other baking powders, add 15 grams of calcium sulfate.

Cool and make up to the original volume with water. Add while stirring, 1 liter of filtered 95% alcohol, either ethyl or methyl, cover and let stand overnight.

By means of a siphon which can be controlled by a pinch-cock, transfer the clear supernatant liquid to a Büchner funnel with 3 layers of filter paper (a suitable size Büchner fits a 9 cm. filter paper). A paper equivalent to C. S. & S. No. 589 Blue Ribbon should be employed, using suction.

To the moist residue remaining in the beaker, add 100 cc. of the acid-alcohol-water mixture. Stir well and let settle. Pour this liquid on the filter. Repeat this operation, using a fresh 100 cc. portion of the acid-alcohol-water mixture. Wash the beaker, residue and filter with 70% alcohol, passing the washings through the filter until the filtrate is nearly free from acid. Discard the filtrate and washings. Wash the filtering flask. Transfer the residue and the filter to the original beaker. Extract the lead sulfate from the residue by using 100 cc. portions of alkaline ammonium acetate solution and by heating to boiling; pass the solution through a new filter using an ordinary funnel. Five extractions are necessary.

Transfer the filtrate, which will measure about 500 cc., to a lipped beaker (see Note 4). Neutralize the glacial acetic acid, using litmus paper as an indicator; then add 10 cc. of glacial acetic acid in excess. Heat nearly to boiling and add 25 cc. of saturated potassium chromate solution. Cover and let stand 48 hours at room temperature, stirring occasionally. Filter through a tared Gooch crucible. Wash well with cold water and dry at 125°C. for at least 45 minutes. Cool in a desiccator and weigh.

The weight of lead chromate multiplied by the factor 0.641 gives the weight of the lead.

NOTES.

1. During the hydrolysis of the starch, do not heat the mixture to a brown color, as this greatly interferes with the filtration.
2. The calcium sulfate is added as a diluent and carrier for the lead sulfate.
3. The alcohol used should be redistilled and kept in glass. Either ethyl or methyl alcohol is efficient.
4. If desired an aliquot (10 cc.) of the alkaline ammonium acetate solution containing the lead may be used colorimetrically[1].
5. A practically complete solution of the calcium sulfate by the alkaline ammonium acetate solution is advisable for the complete solution of the lead sulfate.
6. The Gooch crucible should be prepared with a good felt of purified asbestos fiber and dried at 125°C. for at least 45 minutes.

Three collaborators reported analyses of lead by this method as follows:

[1] *Assoc. Official Agr. Chemists, Methods*, 1920, 285.

TABLE 1.

*Gravimetric determination of lead in baking powder by the Chittick method.*

| COLLABORATOR | LEAD PRESENT | LEAD FOUND |
|---|---|---|
| | *parts per million* | *parts per million* |
| F. B. Carpenter, Virginia-Carolina Chemical Company, Richmond, Va. | 53 | 37<br>38 |
| Ruth Buchanan, Bureau of Chemistry, Washington, D. C. | 53 | 46<br>48<br>50 |
| J. R. Chittick and G. D. Richards, Jaques Manufacturing Company, Chicago, Ill. | 53 | 52<br>55<br>50 |

The results in Table 1 indicate that this method as modified is capable of yielding accurate results.

TABLE 2.

*Electrolytic determination of lead in baking powder by the Corper-Bryan method.*

| COLLABORATOR | LEAD PRESENT | LEAD FOUND |
|---|---|---|
| | *parts per million* | *parts per million* |
| F. B. Carpenter | 53 | 34*<br>38*<br><br>57†<br>62†<br>63†<br>60†<br>60† |
| E. W. Thornton, R. B. Davis Co., Hoboken, N. J. | 53 | 19‡<br><br>36¶<br>25¶ |
| Ruth Buchanan | 53 | 20‡<br>23‡<br>19‡<br>24‡<br>18‡ |
| G. C. Forrester, State Department of Agriculture, E. Lansing, Mich. | 53 | 51 |

* Electrolyzed overnight.
† Electrolyzed 3 days.
‡ Regular method.
¶ One-half neutralized, then other half added.

*Determination of lead by the electrolytic method.*

Samples for the determination of lead by the electrolytic method were the same as those used for lead determination by the gravimetric method.

It was suggested that the collaborators make one determination as directed in the published method, and another by dividing the solution into two equal parts just before neutralizing with ammonia, adding ammonia to half of the solution until incipient precipitation occurs, then adding the other half of the solution and electrolyzing.

That the hydrogen ion concentration of the solution to be electrolyzed may vary widely was shown by Forrester, who reported as follows:

A liter of aqueous lead chloride solution containing 0.135 gram of the dry salt equivalent to 0.1575 gram of lead chromate was prepared. 50 cc. of this solution then contained lead equivalent to 0.0078 gram of lead chromate.

Using 50 cc. of this solution and with varying quantities of concentrated hydrochloric acid (sp. gr. 1.19) and distilled water to a volume of 400 cc., 8 samples were prepared and electrolyzed overnight (16 hours) with a current of 0.15 to 0.30 amperes. The deposit of lead was dissolved in nitric acid and precipitated as lead chromate with the following results:

TABLE 3.

*Lead recovered using varying amounts of acid.*

| VOLUME OF STANDARD | VOLUME OF ACID | TOTAL VOLUME | LEAD CHROMATE | THEORY |
|---|---|---|---|---|
| *cc.* | *cc.* | *cc.* | *gram* | *gram* |
| 50 | 0. | 400 | 0.0077 | 0.00787 |
| 50 | 5. | 400 | 0.0073 | 0.00787 |
| 50 | 10. | 400 | 0.0081 | 0.00787 |
| 50 | 10. | 400 | 0.0077 | 0.00787 |
| 50 | 15. | 400 | 0.0077 | 0.00787 |
| 50 | 25. | 400 | 0.0075 | 0.00787 |
| 50 | 50. | 400 | 0.0076 | 0.00787 |
| 50 | 75. | 400 | 0.0081 | 0.00787 |

Based upon these eight determinations, the concentration of acid within limits encountered in working with samples had no influence outside the limits of experimental error on the quantitative deposition of lead.

Since a quantity of 1920 collaborative samples of baking powder for lead determination was still on hand (it is assumed that these samples contained 50 parts per million of lead), the investigation was extended to ascertain whether any ingredient in a baking powder would interfere with the foregoing generalization. For this purpose samples were prepared as directed using 75 cc. of concentrated hydrochloric acid and, with the different samples, varying amounts of ammonium hydroxide were added to partially neutralize the acid present.

The average weight of lead chromate, 0.0082 gram, is equivalent to 0.00525 gram of lead which, from a 100-gram sample, indicates 52.5 parts of lead per million. Therefore, it would appear that the Corper-Bryan method without attention to concentration of acid, other than that it be sufficient to prevent precipitation of salts present, is quite satisfactory for all practical purposes.

The investigation made by Forrester shows that the hydrogen ion concentration of the solution may vary widely without affecting the complete deposition of lead upon the electrodes. It would seem, therefore, that other considerations govern the completeness of the deposition

of the lead, and it is thought that a further study of these control conditions should be made before the method is adopted.

TABLE 4.

*Lead recovered using varying amounts of ammonia.*

| WEIGHT OF SAMPLE | ORIGINAL ACID | ACID NEUTRALIZED BY AMMONIUM HYDROXIDE | LEAD CHROMATE |
|---|---|---|---|
| *grams* | *cc.* | *cc.* | *gram* |
| 100 | 75 | 0 | 0.0084 |
| 100 | 75 | 8 | 0.0076 |
| 100 | 75 | 15 | 0.0085 |
| 100 | 75 | 35 | 0.0084 |
| 100* | 75 | 50 | 0.0041 |

* A heavy precipitate, due to excess ammonia, interfered and result was not taken into consideration.

Carpenter reports that the period of time stated in the Corper-Bryan method is insufficient to obtain a complete deposition of the lead. He found it necessary to electrolyze for 2 or 3 days in order to get all the lead out of the solution.

While the collaborative results as given in Table 2 do not show close agreement, some analysts obtained results close to theory, and it is believed that further study will reveal the exact conditions necessary to get complete deposition of the lead.

## NEUTRALIZING STRENGTH OF BAKING ACIDS.

For the study of the neutralizing strength of baking acids, five methods were submitted to the collaborators, some of which had to be modified to meet the requirements of the different acids. Samples of the baking acids—monocalcium phosphate, sodium aluminium sulfate and a mixture of 58 per cent of monocalcium phosphate and 42 per cent of sodium aluminium sulfate—were furnished through the courtesy of Thornton.

Directions for determining the neutralizing strength follow:

### *Method A.*

Weigh out 0.8401 gram of phosphate into a 3A casserole, add 25 cc. of water and stir a moment. Add exactly 90 cc. of 0.1N sodium hydroxide. (In case of S. A. S. add exactly 110 cc. of 0.1N sodium hydroxide.) Bring to a boil; boil for 1 minute; add 1 drop of 1% phenolphthalein solution and titrate while still boiling hot with 0.2N hydrochloric acid. The end point is obtained when pink color due to indicator has all but disappeared and does not return in one minute.

#### CALCULATION.

$90-2\times$ (cc. standard hydrochloric acid used) = neutralizing strength of 100 parts of phosphate in terms of bicarbonate of soda.

### *Method B.*

Weigh out 0.84 gram of the phosphate into a 250 cc. beaker and add 125 cc. of water and 10–15 drops of a 1% solution of phenolphthalein indicator. Titrate with 0.5N

sodium hydroxide to a faint pink; heat to boiling; boil for 1 minute and continue titrating while hot till a permanent pink is reached. The total reading, multiplied by 5, equals the neutralizing value in terms of parts sodium bicarbonate per 100 parts of phosphate.

*Method C.*

Weigh 1 gram of the sample into a 300 cc. flask, add 100 cc. of water and 1.0–1.5 cc. of a 1% solution of phenolphthalein and shake well. Titrate with N sodium hydroxide, adding 1 cc. of N sodium hydroxide in excess. Boil 1 minute. Titrate, while hot, to acid with N sulfuric acid. Titrate finally (hot) to permanent pink. Add together the amounts of N sodium hydroxide used and from this sum subtract the amount of N sulfuric acid used. The difference in cc. times the factor 0.084 times 100 equals the neutralizing value of 100 parts of phosphate in terms of bicarbonate of soda.

*Method D.*

Weigh 0.840 gram of phosphate into a 3A casserole and add 75 cc. of saturated salt (NaCl) solution. Titrate after adding a 1% solution of phenolphthalein until faint pink with 0.5N sodium hydroxide. Then add an excess of 0.5N sodium hydroxide to bring the total number of cc. up to 20, boil the solution for 2 minutes and allow to cool. (In case of the S. A. S., a total of 24 cc. of 0.5N sodium hydroxide is used and for the mixture of phosphate and S. A. S., a total of 18 cc.) Add 80 cc. of 0.2N hydrochloric acid. (For S. A. S., add 10 cc. of 0.2N hydrochloric acid and for mixture of S. A. S. and phosphate, add 7 cc. of 0.2N hydrochloric acid.) Titrate the solution with 0.5N sodium hydroxide.

CALCULATION.

The cc. of 0.1N sodium hydroxide (first titration) equals the "cold test" or parts of bicarbonate per hundred parts of phosphate in the cold. The cc. of 0.1N sodium hydroxide (total used) − the cc. of 0.1N hydrochloric acid = the "total strength" or parts of bicarbonate per hundred parts of phosphate.

*Method E.*

Weigh 0.84 gram of phosphate and 1 gram of starch in a No. 4 casserole. Add 45 cc. of 0.2N sodium hydroxide and stir well. (In case of the S. A. S. add 50 cc. of 0.2N sodium hydroxide.) Add 100 cc. of neutral sodium sulfate (250 grams of crystallized sodium sulfate per liter) and 1 drop of 1% solution of phenolphthalein. Heat to a brisk boil. Titrate while hot with 0.2N hydrochloric acid till pink color disappears and does not return on standing 1 minute.

CALCULATION.

(45 − number of cc. of hydrochloric acid used) × 2 = neutralizing value of 100 parts of phosphate in terms of bicarbonate of soda. In order to be sure of neutrality of the sodium sulfate solution and starch, it is best to run a blank, using 1 gram of starch and 100 cc. of neutral sodium sulfate solution.

The collaborative results on the neutralizing values indicate that any of the five methods outlined may be used successfully in the case of sodium aluminium sulfate. The results on the combination of sodium sulfate and monocalcium phosphate are somewhat lower than those on the monocalcium phosphate alone. Methods A and C appear to be the most satisfactory. The principal difficulty with these methods is in getting a definite end point when phenolphthalein is used as the indi-

TABLE 5.

*Collaborative results on neutralizing values.*

| COLLABORATOR | METHOD | PHOSPHATE | PHOSPHATE AND S. A. S.* | S. A. S. |
|---|---|---|---|---|
| F. B. Carpenter | A | 76 | 75 | 103 |
| | B | 79 | 73 | 98 |
| | C | 88 | 74 | 103 |
| | D | 85 | 82 | 105 |
| | E | 83 | 78 | 102 |
| A. H. Fiske, Rumford Chemical Works, Providence, R. I. | A | 82 | 80 | 101 |
| | B | 75 | 70 | 100 |
| | C | 74 | 74 | 100 |
| | D | 94 | 84 | 100 |
| | E | 86 | 81 | 105 |
| L. D. Mathias, Victor Chemical Works, Chicago, Ill. | A | 82 | .. | ... |
| | B | 77 | 70 | 100 |
| | C | 79 | .. | ... |
| | D | .. | .. | ... |
| | E | 91 | .. | ... |
| E. W. Thornton | A | 79 | 77 | 100 |
| | B | 75 | 70 | 100 |
| | C | 75 | 71 | 100 |
| | D | 81 | 78 | 101 |
| | E | 83 | 82 | 96 |
| W. E. Stokes, Royal Baking Powder Co., New York, N. Y. | A | 90 | .. | ... |
| | B | 72 | .. | ... |
| | C | 70 | .. | ... |
| | D | 85 | .. | ... |
| | E | 81 | .. | ... |
| Axel Malmstrom, Wilckes-Martin-Wilckes Co., Camden, N. J. | A | 81 | 80 | 103 |
| | B | 76 | 75 | 100 |
| | C | 76 | 74 | 103 |
| | D | 88 | 80 | 103 |
| | E | 84 | 76 | 105 |
| R. S. Dixon, Provident Chemical Works, St. Louis, Mo. | A | 79 | 78 | 104 |
| | B | 76 | 72 | 103 |
| | C | 71 | 71 | 100 |
| | D | 85 | 83 | 102 |
| | E | 81 | 79 | 101 |
| Dorothy Gaylord, Larkin Co., Buffalo, N. Y. | A | 77 | 77 | 100 |
| | B | 76 | 71 | 101 |
| | C | 70 | 70 | 101 |
| | D | 90 | 83 | 104 |
| | E | 87 | 80 | 105 |
| Ruth Buchanan | A | 79 | 79 | 101 |
| | B | 72 | 70 | 95 |
| | C | 73 | 71 | 101 |
| | D | 84 | 83 | 98 |
| | E | 83 | 81 | 101 |

* 58% of monocalcium phosphate and 42% of sodium aluminium sulfate.

cator. It has been suggested that experiments be made using other indicators or a combination of indicators in order to secure a more definite end point and thus reduce, to some extent, variation in results due to the personal element.

## CARBON DIOXIDE IN BAKING POWDER.

(The Robinson Method.)

Since presenting his paper, "Determination of Total Carbon Dioxide in Baking Powder", C. S. Robinson has worked out a method of determining also the residual carbon dioxide by using the same volumetric apparatus. This apparatus is a modification of the Van Slyke carbon dioxide apparatus. By its use one can read directly the volume of gas liberated from a sample of baking powder. Only a limited number of laboratories are supplied with this apparatus and hence only a few collaborators could be secured. The results obtained compare favorably with those obtained by the official Knorr method.

TABLE 6.

*Comparative results on the determination of carbon dioxide.*

| COLLABORATOR | TOTAL CARBON DIOXIDE | | RESIDUAL CARBON DIOXIDE | |
|---|---|---|---|---|
| | Robinson method | Knorr method | Robinson method | Knorr method |
| | *per cent* | *per cent* | *per cent* | *per cent* |
| C. S. Robinson..... | 16.69 | 16.67 | 1.31 | 1.39 |
| G. C. Forrester..... | 16.21 | ..... | 0.67 | .... |
| Ruth Buchanan..... | 16.30 | ..... | 1.03 | .... |
| D. B. Scott, Bureau of Chemistry, Washington, D. C..... | ..... | 15.92 | .... | 0.82 |

## RECOMMENDATIONS.

It is recommended—

(1) That the modified Chittick method be adopted as a tentative method.

(2) That a further study of the control conditions for the completeness of the deposition of the lead be made before the method is adopted.

(3) That the use of different indicators or a combination of indicators be studied in connection with the determination of the neutralizing strength of phosphate used in the manufacture of baking powder.

(4) That further study be given to the determination of fluorine in baking powder.

(5) That a further study be made of the volumetric methods of determining carbon dioxide in baking powder.

A paper, entitled "The Determination of Carbon Dioxide in Baking Powder"[1], was submitted by C. S. Robinson and Selma L. Bandemer.

[1] *J. Ind. Eng. Chem.*, 1922, **14**: 119.

# REPORT ON FLUORIDES IN BAKING POWDER.

By JAMES K. MORTON (Bureau of Chemistry, Washington, D. C.), *Associate Referee.*

In making a large number of fluorine determinations on rat samples by the Wagner-Ross[1] method, it became necessary to check this method. Carefully selected samples of fluorspar, sodium fluoride (Kahlbaum) and sodium fluosilicate were used, and about forty analyses were made. It was found impossible to secure consistent results. The recovery of fluorine varied from 60 to 95 per cent, the average being about 80 per cent. This discrepancy was unexplainable, and no data have since been found that would substantiate the results given by the authors in their original paper.

A number of modifications of the apparatus and of manipulation were tried. Air was substituted for carbon dioxide gas and the style of the digestion flask modified without changing the result in any material way.

Although the time was too short to send samples for collaborative work, a number of analyses of baking powders were made.

The determination of fluorine by this method presents a number of difficulties. In preparing the sample for analysis it is very important that the organic matter be driven off; at the same time it must not be heated high enough to volatilize any fluorine present. The presence of carbon in the digestion flask gives rise to sulfur dioxide. In passing through the system this gas is not wholly retained by the chromic acid solution, but it passes into the absorption tube to form a little sulfuric acid, which is not removed by boiling and which gives high results. The presence of sulfuric acid must be tested for with barium chloride, and if any is found it must be carefully estimated by comparison with known standards and proper allowance made.

The qualitative test for fluorine is extremely sensitive. Its presence in the digestion flask is indicated by a characteristic scum which forms on the surface of the sulfuric acid when the flask is slightly heated. An experienced observer can detect as low as 0.005 per cent of fluorine. If any silicon fluoride comes over into the absorption tube, 0.005 per cent can be easily detected by the deposit of silicic acid on the delivery tube at the point of contact of the gas with water.

The results found by analyzing five samples of baking powder are given in the following table:

[1] *J. Ind. Eng. Chem.*, 1917, 9: 1116.

*Determination of fluorides in baking powders.*

| SAMPLE NUMBER | DESCRIPTION | WEIGHT TAKEN | FLUORINE ADDED | 0.1N POTASSIUM HYDROXIDE | FLUORINE IN SAMPLE | FLUORINE IN SAMPLE | FLUORINE RECOVERED | QUALITATIVE TEST |
|---|---|---|---|---|---|---|---|---|
| | | *gram* | *gram* | *cc.* | *gram* | *per cent* | *per cent* | |
| 1 | Commercial alum-phosphate baking powder.. | 20 | none | 0.6 | 0.00114 | 0.0055 | ..... | Very slight |
| | | 20 | none | 0.6 | 0.00114 | 0.0055 | ..... | Very slight |
| 2 | No. 1+0.02 per cent of fluorine as calcium fluoride..... | 20 | 0.00514* | 2.3 | 0.00437 | 0.0218 | 85.0 | Positive |
| | | 20 | 0.00514* | 1.8 | 0.00342 | 0.0171 | 66.5 | Positive |
| 3 | Commercial phosphate baking powder..... | 20 | none | 0.0 | none | none | ..... | None |
| 4 | Commercial alum baking powder, low in phosphate..... | 20 | none | 0.0 | ..... | ..... | ..... | None |
| 2† | | 20 | 0.00514* | 2.6 | 0.00494 | 0.0247 | 96.1 | Positive |
| | | 20 | 0.00514* | 2.8 | 0.00532 | 0.0266 | 103.5 | Positive |
| | | 20 | 0.00514* | 2.7 | 0.00513 | 0.0257 | 99.8 | Positive |
| | | 20 | 0.00514* | 2.5 | 0.00475 | 0.0237 | 92.4 | Positive |
| | | 40 | 0.01028* | 5.0 | 0.0095 | 0.0237 | 92.4 | Positive |
| 5† | Equal parts of Samples 1 and 2 ..... | 40 | 0.00628* | 2.5 | 0.00475 | 0.0119 | 75.0 | Positive |

* Fluorine found in No. 1 added.
† Manipulation slightly modified.

From the table it can readily be seen that when the sample contains a very low percentage of fluorine the results of the determination are good. By using a slight modification the recovery of fluorine approximates 100 per cent. The loss of fluorine is traced to the volatilization of hydrofluoric acid which passes over into the traps containing sulfuric acid before it can react with the silica in the digestion flask. A slight modification in the procedure made it possible to retain the hydrofluoric acid in the digestion flask in order that it might react more completely with the silica present to form silicon fluoride. This gave a more complete recovery of fluorine and more consistent results.

The following modification of the Wagner-Ross method for the determination of fluorine in baking powder is proposed:

Weigh 20 grams of baking powder in an evaporating dish 10½ cm. in diameter, add sufficient water to make a fairly thick paste and place on the steam bath until most of the water has been driven off. Dry at 110°C. for 1 hour. Place in a muffle furnace and heat slowly with the furnace door open until the material has charred. Close the door of the furnace and heat to a very low red heat, not over 500°C., until most of the carbon has been burned off, leaving a grayish white ash. Allow it to cool. Powder the material and add just enough water to moisten the ash, reignite and repeat this operation until an ash free of carbon is obtained.

Cool in a desiccator. Place the ash, together with 5 grams of silica (i. e. quartz flour) and 5 grams of anhydrous copper sulfate in a digestion flask (Pyrex) of 120 cc. capacity, having a single trap and reflux tube at the outlet, and thoroughly mix the contents of the flask.

Connect the flask in its position in the train. Force 50 cc. of 98.5 per cent sulfuric acid over into the digestion flask from a 50 cc. Erlenmeyer connected just behind the digestion flask, in the train. Allow a portion of the acid to run into the trap by slightly tilting it. Pass air, thoroughly dried over sulfuric acid, through the train very slowly, not over 1 bubble per second. Allow the flask, without shaking, to remain in the cold for not less than ½ hour. Apply heat very slowly, bringing the acid to boiling in about 1 hour, and boil freely for 10 minutes. Remove the flame and continue to pass air through the system for ½ hour longer. Increase the rate of flow of the air to about 3 bubbles per second. Proceed as in the original method.

## RECOMMENDATIONS.

It is recommended—

(1) That the Wagner-Ross method with modifications be submitted for further study during the coming year.

(2) That the Wagner-Ross method with modifications be continued as a tentative method.

The meeting adjourned at 5 p. m. for the day.

## FIRST DAY.

# MONDAY—AFTERNOON SESSION.—Continued.

### DRUG SECTION.

## REPORT ON DRUGS.

By George W. Hoover (U. S. Food and Drug Inspection Station, Transportation Building, Chicago, Ill.), *Referee.*

In accordance with the action of the association last year, your referee appointed a number of associate referees to develop or select suitable methods for the analysis of important drugs. Collaborative work was conducted, and some preliminary reports on methods were made by the associate referees. It is believed that the association should continue the work on drugs under the same general plan as long as it is considered that satisfactory progress is being made.

A number of other subjects should receive attention as soon as possible. The associate referees should, if possible, secure the services of men who are familiar with the specific subject assigned and who will take special interest in the work. This is difficult oftentimes owing to lack of interest or time. A large number of analysts are not necessary; in fact, a few collaborators giving serious attention to a specific subject are more to be desired. Another point to be considered is the importance of proper selection of subjects for study. Often, after considerable effort, it has been found that work for which there is much need has been neglected for that of minor importance. Suggestions of the associate referees and collaborators should serve as guides in determining the subjects upon which it is most desirable to conduct work and the general manner of procedure. The associate referees and collaborators are to be congratulated on the quality and unusual amount of work that has been done during the past year.

## QUALITATIVE AND QUANTITATIVE ANALYSIS OF ARSPHENAMINE (SALVARSAN) AND NEOARSPHENAMINE (NEOSALVARSAN)[1].

By George W. Hoover and Chris K. Glycart (U. S. Food and Drug Inspection Station, Transportation Building, Chicago, Ill.).

Arsphenamine and neoarsphenamine are used extensively at the present time. Although in former years the supply of these products came from foreign countries, at the present time it is principally of

[1] Presented by Mr. Glycart.

domestic origin. However, it is being imported to some extent and no doubt the importations will increase in the future as business relations with foreign countries become adjusted. It is fully appreciated that in the examination of these products it is necessary to take into consideration both the chemical analysis for arsenic content and the biological examination for toxicity. In this work only the qualitative and quantitative chemical analyses have been considered.

All the collaborators—H. Engelhardt and R. I. Grantham, Sharp and Dohme, Baltimore, Md.; George W. Raiziss, The Dermatological Research Laboratories, 1720 Lombard St., Philadelphia, Pa.; and F. W. Heyl, The Upjohn Co., Kalamazoo, Mich.—have had wide experience in handling these products.

Investigators of this subject consider the modified Lehmann method[1] one of the most suitable for the determination of arsenic. The reasons given—that its use by different collaborators on the same sample gives satisfactory results; that it is rapid; and that aside from the collaborative work given in this report, it has won the support of the chemists of the United States Public Health Service—entitle this method to serious consideration. One of the collaborators, however, states that the gravimetric method that he employs is very satisfactory.

A sufficient number of samples of arsphenamine and neoarsphenamine manufactured from the same batches were secured to supply the collaborators with samples of uniform composition. The following directions were submitted for conducting the work:

*Qualitative Tests for Arsphenamine (Salvarsan).*

3 – diamino 4 – dihydroxy – 1 – arsenobenzene dihydrochloride corresponding to 31.57% arsenic.
$HClNH_2 . OH . C_6H_3 . As : As . C_6H_3 . OH . NH_2 HCl + 2H_2O$.

PHYSICAL PROPERTIES.

Arsphenamine is a pale yellow powder, unstable in moist air. It is soluble in water, 1 to 5 parts, methyl alcohol 1 to 3 parts, and only slightly soluble in ether. The aqueous solution is greenish yellow, and it reacts strongly acid to litmus. The moisture content is not more than 7.6% when dried in an atmosphere of hydrogen at 105°C.

CHEMICAL PROPERTIES.

An aqueous solution of arsphenamine (1 to 100) yields no precipitate with dilute mineral acids, with the exception of sulfuric acid (distinction from neoarsphenamine).

The addition of sodium hydroxide T. S. yields a precipitate which is soluble in excess of the reagent.

Heated with alkaline solution of potassium permanganate, ammonia is liberated.

Mayer's reagent produces a heavy orange-yellow precipitate.

Ferric chloride solution produces a brownish violet color, turning turbid.

[1] *Apoth.-Ztg.*, 1912, 27: 545.

Silver nitrate solution added drop by drop produces a dark red precipitate which changes to black precipitate.

Reinsch test is positive.

Hydrogen sulfide produces no precipitate even after addition of hydrochloric acid and warming.

*Qualitative Tests for Neoarsphenamine (Neosalvarsan).*

Sodium 3 – diamino – 4 – dihydroxy – 1 – arseno-
benzene – methylene – sulfoxylate.
$NH_2 . OH . C_6H_3 . As : As . C_6H_3 . OH.NH(CH_2O) OSNa$.
Mixed with inert inorganic salts.

### PHYSICAL PROPERTIES.

Neoarsphenamine is a lemon-yellow powder, unstable in moist air, turning to a reddish brown color. It is readily soluble in water but only slightly soluble in alcohol or ether. The aqueous solution is neutral to litmus. On exposure to air the solution rapidly becomes dark brown.

### CHEMICAL PROPERTIES.

A freshly prepared aqueous solution of neoarsphenamine (1 to 100) yields a tardy precipitate on addition of dilute mineral acids (distinction from arsphenamine).

The addition of 10% sodium hydroxide solution produces no precipitate (distinction from arsphenamine).

Solution of alkali carbonates produces no precipitate (distinction from arsphenamine).

Mayer's reagent produces no precipitate until the solution is acidified with dilute hydrochloric acid (distinction from arsphenamine, which yields a precipitate directly).

Ferric chloride solution produces a violet color, turning to dark red.

Silver nitrate solution produces a brown color, quickly forming a black precipitate.

If 5 cc. of dilute hydrochloric acid is added and the mixture heated, the irritating odor of sulfur dioxide will be evolved (distinction from arsphenamine).

*Quantitative Determination of Arsenic in Arsphenamine and Neoarsphenamine.*

### REAGENTS.

(a) 3% hydrogen peroxide solution.
(b) Oxalic acid solution—Dissolve 1 gram in water, make to 100 cc.
(c) C. P. potassium iodide.
(d) C. P. potassium permanganate (finely ground).
(e) Potassium permanganate solution—Dissolve 1 gram in water, make to 100 cc.
(f) 0.1N sodium thiosulfate solution.

### DETERMINATION.

Dissolve 0.2 gram in 5 cc. of 10% sulfuric acid by volume in a 200 cc. Erlenmeyer flask, fitted with a ground-glass stopper. A blank is conducted, using the reagents under the same conditions, and the amount of 0.1N sodium thiosulfate consumed is deducted. Add 1 gram of finely powdered potassium permanganate in small portions, mix thoroughly and allow to stand for 10 minutes. Add 10 cc. of concentrated sulfuric acid in 2 cc. portions. Shake thoroughly after each addition. Allow to digest 10 minutes, rotating the flask frequently during this period. Add 5 to 10 cc. of hydrogen peroxide solution drop by drop until the brown color disappears. To remove excess of peroxide, add 25 cc. of water, boil gently for 10 minutes and carefully add a few drops of a 1% solution of potassium permanganate until the pink color is just permanent. To remove excess of permanganate, add a drop or two of oxalic acid solution. Dilute with 50 cc.

of water. When the solution is cool, add 2 grams of potassium iodide, stopper flask tightly and let stand for 1 hour in a cool place. Titrate the liberated iodine with 0.1N sodium thiosulfate, omitting the use of starch indicator.

1 cc. of 0.1N sodium thiosulfate is equivalent to 0.00375 gram of arsenic.

The arsenic content of arsphenamine should not be below 30 or above 32%.

The arsenic content of neoarsphenamine should not be below 18 or above 20%.

*Determination of Arsenic in Arsphenamine and Neoarsphenamine.*

| COLLABORATORS | ARSPHENAMINE SAMPLE A-1 | ARSPHENAMINE SAMPLE A-1 | NEOARSPHENAMINE SAMPLE N-2 | NEOARSPHENAMINE SAMPLE N-2 |
|---|---|---|---|---|
| | *per cent* | *per cent* | *per cent* | *per cent* |
| H. Engelhardt | 29.90 | 30.00* | 18.1 | 18.56* |
| R. I. Grantham | 29.50 | ..... | 18.0 | ..... |
| F. W. Heyl | 30.69 | ..... | 18.48 | ..... |
| | 30.79 | ..... | 18.30 | ..... |
| C. K. Glycart | 30.00 | 30.00† | 18.53 | ..... |
| | 30.07 | 29.45* | 18.48 | ..... |
| George W. Raiziss | 28.08* | 30.21† | 16.86* | 19.09† |

*After digestion with permanganate, the liberated iodine was discharged by 2% sodium sulfite solution and then titrated with 0.1N iodine.

†Sample was dissolved in 5 cc. of 15% sulfuric acid.

## COMMENTS BY COLLABORATORS.

*H. Engelhardt.*—In regard to the volumetric process we believe that the method has one disadvantage in that a blank test has to be made. In carrying out this test, we used in one case 0.45 cc. and in another 0.9 cc. of 0.1N thiosulfate solution for titrating the iodine. This difference may be due to the fact that in the latter case the aqueous acid solution was more concentrated than in the former and that consequently a more concentrated acid was allowed to act on the potassium iodide, by which more iodine was liberated. Such discrepancies could probably be obviated by making up the final liquid to be titrated to a definite volume, say 75 cc., instead of adding 50 cc. of water to an indefinite volume of liquid. Furthermore, the oxidation might be carried out with the aid of heat on a steam bath and the reduction of the arsenic acid to arsenious acid at 40–50°C., by which the time consumed for the latter could be reduced from 1 hour to about 10 minutes. Grantham then applied an assay method which we are using in our laboratory for assaying organically combined arsenic products. This method differs from the one submitted in that the iodine liberated by the reduction of the arsenic acid is not titrated directly, but the solution is decolorized by the careful addition of a 2% solution of sodium sulfite, made slightly alkaline with caustic soda solution, rendered slightly acid with hydrochloric acid and after the addition of 5 grams of sodium bicarbonate is titrated with 0.1N iodine solution. No blank is necessary in this test.

In regard to the qualitative tests, it is considered that the reaction with silver nitrate, both in the case of arsphenamine and of neoarsphenamine, is very indistinct and should be revised.

*George W. Raiziss.*—The method for the quantitative determination of arsenic, I find, approaches the Lehmann method. We are using this method for our analysis, but our procedure differs in the following detail: Take up 200 mgs. of the drug with 5 cc. of 15% sulfuric acid. The gravimetric determination of arsenic in these samples has not been made because we feel that the modified Lehmann method we are using is

giving exactly the same results as the gravimetric determination of arsenic. We ran comparative analyses of a great number of samples and always found a close agreement.

Raiziss gave attention to the ratio of arsenic to nitrogen, and from his conclusions it appears that this ratio is important in judging the purity of the products. It would seem advisable, therefore, for the associate referee to develop methods to determine the ratio of arsenic to nitrogen. In the future study of this subject it may be desirable to consider the ratio of other component elements of arsphenamine and neoarsphenamine.

## RECOMMENDATIONS.

It is recommended—

(1) That the qualitative and quantitative methods submitted herewith be adopted by the association as tentative methods, and that they be further studied during the next year with a view to their official adoption.

(2) That the modification suggested by Engelhardt, which provides for digestion with potassium permanganate, the addition of potassium iodide, the discharge of liberated iodine by the use of sodium sulfite solution and final titration with 0.1N iodine, be studied during the next year.

(3) That during the next year the associate referee study and devise methods to determine the ratio of arsenic to nitrogen in arsphenamine and neoarsphenamine.

As this is the first report made to the association on the subject of arsphenamine and neoarsphenamine, the following references to some of the most important articles on the chemistry of these and allied products are given:

Boon. J. Soc. Chem. Ind., 1914, 33: 1187.
Engelhardt and Winters. J. Am. Pharm. Assoc., 1915, 4: 1468.
Lehmann. Apoth.-Ztg., 1912, 27: 545.
New and Nonofficial Remedies, 1917, 43.
Puckner and Hilpert. J. Am. Med. Assoc., 1910, 55: 2314.
Raiziss and Falkov. J. Biol. Chem., 1921, 46: xliv.
Raiziss and Proskouriskoff. Arch. Dermat. and Syph., 1920, 2: 280.
U. S. Public Health Service Reports, 1918, 1003.

# REPORT ON THE DETERMINATION OF ALCOHOL IN DRUG PRODUCTS.

By A. G. MURRAY (Bureau of Chemistry, Washington, D. C.), *Associate Referee.*

The following method was submitted to several collaborators:

## TENTATIVE METHOD FOR THE DETERMINATION OF ALCOHOL IN DRUG PRODUCTS.

I.—Introduce into a distilling flask of about 200 cc. capacity a measured volume of the preparation (which has previously been brought to a temperature of 20°C.) not exceeding 100 cc. and containing not more than 10 cc. of absolute alcohol. Add sufficient water to bring the total volume to 75–100 cc. Connect the flask with a suitable condenser, to the lower end of which is attached an adapter which extends through the neck and into the bulb of a 50 cc. graduated flask. Distil at such a rate that there is no danger of loss of alcohol. (The proper rate of distillation will depend upon many factors, such as the temperature of the condenser water, the type and size of condenser, etc.) Continue the distillation until the flask is filled nearly to the mark. If the distillate consists of a mixture of alcohol and water only, place the flask for half an hour in a bath maintained at 20°C., fill to the mark with distilled water at 20°C., mix thoroughly and determine the specific gravity and refractivity at convenient temperatures and ascertain the proportion of alcohol by volume in the distillate from the reference tables[1], Nos. 7 and 8. From the percentage of alcohol found in the distillate calculate the percentage in the preparation by multiplying by the proper factor.

COMMENTS.

Instead of the usual form of distilling flask the referee uses an ordinary round-bottom flask connected to a vertical condenser of the spiral type by means of a spray trap. Is there any advantage?

Can the alcohol from volumes as great as 100 cc. be safely distilled into 50 cc. where the distillate will contain as much as 20% alcohol by volume? Can any higher concentration be safely permitted? Would the addition of salt or sodium sulfate to the contents of the distilling flask to raise the boiling point be of any advantage?

Is it necessary or desirable to have 1 or 2 cc. of water in the receiving flask to dilute the first runnings and prevent loss by evaporation?

Is it necessary or desirable to immerse the receiving flask in a cooling bath?

II.—If *frothing* due to saponin occurs the glucoside may be hydrolized by adding a little dilute sulfuric or hydrochloric acid to the contents of the distilling flask and boiling over a low flame. As the frothing ceases, increase the heat and complete the distillation as usual.

III.—The distilling flask should be so protected that no charring of non-volatile organic matter occurs. If necessary an oil or glycerine bath should be used to heat the contents of the distilling flask.

COMMENT.

What, if any, special precautions do you take in cases where preparations contain a large amount of extractives?

IV.—If the preparation contains glycerine the volume taken for the determination of alcohol must be such that the residue remaining in the distilling flask at the end of

[1] *Assoc. Official Agr. Chemists, Methods*, 1920, 345.

the distillation shall contain not more than 50% of glycerine, or the distillate must be redistilled.

V.—If the preparation contains iodine reduce with zinc dust or sodium thiosulfate. If sodium thiosulfate is used a few drops of sodium hydroxide solution should be added to prevent the distillation of sulfur.

COMMENT.

Does the necessity for not heating a mixture of alcohol, iodine and alkali, resulting in the formation of iodoform at the expense of alcohol, need to be specifically mentioned in this connection?

VI.—If the preparation contains a volatile acid neutralize with sodium hydroxide. If it contains a volatile base neutralize with dilute sulfuric acid. If it contains both a volatile acid and a volatile base neutralize first with dilute sulfuric acid and distil about 50 cc.; neutralize the distillate with sodium hydroxide and redistil.

VII.—If the preparation contains acetone, camphor, chloroform, ether, or a volatile oil, dilute the portion to be distilled, if necessary, so that the alcohol content is not more than 25% by volume; saturate with common salt and shake in a separator with about 15 cc. of petroleum ether. After the liquids have completely separated draw off the lower alcoholic salt solution into a second separator and repeat the extraction with about 15 cc. of petroleum ether. Draw off the lower alcoholic salt solution into the distilling flask. Wash the two portions of petroleum ether successively with 10 cc. of saturated salt solution and add the aqueous layer to the contents of the distilling flask. Distil and continue as in Procedure I. If the character of the preparation is such as to render difficult the shaking out with petroleum ether make a preliminary distillation as directed, saturate the distillate with salt, shake out with petroleum ether, and redistil as directed.

COMMENT.

How completely is acetone removed by this process? In view of the permitted use of alcohol denatured with acetone for the manufacture of preparations for external use, this detail is likely to be of practical importance.

VIII.—If the preparation contains chloral hydrate add sufficient concentrated sodium hydroxide solution to render the solution 0.5N alkali. Stopper and allow to stand 10 minutes. Shake out with petroleum ether and proceed as directed under Procedure VII.

COMMENT.

The efficacy of this procedure needs experimental verification.

IX.—If it is desired to test the distillate for the presence of methyl alcohol use Deniges' method, as follows: Dilute with water the volume of the distillate which contains about 0.1 cc. of alcohol to about 4 cc., add 1 cc. of 5% potassium permanganate solution and 0.2 cc. of concentrated sulfuric acid. After 3 minutes add a few drops of a cold saturated solution of oxalic acid. When the liquid has become colorless or pale yellow add 1 cc. of concentrated sulfuric acid, mix and cool somewhat. To the colorless liquid add 5 cc. of Schiff's reagent (prepared by adding 100 cc. of a 0.01% solution of magenta to 2 cc. of a saturated solution of hydrogen sodium sulfite and after 5 minutes adding 2 cc. of concentrated hydrochloric acid). The appearance of a violet color after some minutes indicates the presence of methyl alcohol.

COMMENT.

The author claims that the method is capable of detecting 1 part of methyl alcohol in 1000 parts of ethyl alcohol.

X.—If the preparation contains methyl alcohol proceed as directed under **17** or **18** of the official methods[1], or as follows:

From the specific gravity ascertain the content of total alcohol in terms of grams per 100 cc., using the tables for ethyl alcohol. Denote the result by $a$.

To a *cold* mixture of 50 cc. of approximately 0.5N potassium dichromate solution, 25 cc. of water and 50 cc. of concentrated sulfuric acid contained in a 250 cc. graduated flask add an accurately measured aliquot of the distillate (not exceeding 25 cc.) containing not more than 0.2 gram total alcohol. Mix and allow to stand at room temperature for 24 hours. Dilute nearly to the mark with water, cool to room temperature, make up to volume and mix thoroughly. To 50 cc. of the mixture add 100 cc. of water and 15 cc. of a 10% solution of potassium iodide. Titrate with 0.1N sodium thiosulfate, using starch as an indicator. A blank control is run at the same time omitting the alcohol. The difference between the amounts of thiosulfate solution used in the two titrations multiplied by 5 gives the amount corresponding to the alcohol taken. Calculate the number of cc. of 0.1N thiosulfate corresponding to 100 cc. of the distillate. Denote the result by $b$.

Since the bichromate solution oxidizes ethyl alcohol to acetic acid and methyl alcohol to carbon dioxide

1 gram of ethyl alcohol is equivalent to 868.8 cc. of 0.1N thiosulfate, and

1 gram of methyl alcohol is equivalent to 1248.5 cc. of 0.1N thiosulfate.

If $x$ = Number of grams of ethyl alcohol per 100 cc. distillate and $y$ = Number of grams of methyl alcohol per 100 cc. distillate,

$$x+y=a$$
$$868.8\,x + 1248.5\,y = b.$$

From these equations

$$x = \frac{1248.5\,a - b}{379.7} \text{ and}$$

$$y = \frac{b - 868.8\,a}{379.7}.$$

If a higher degree of accuracy is necessary correction must be made for the slight difference in specific gravities of aqueous methyl[2] and ethyl alcohols.

COMMENT.

Is there any necessity for including the *chemical* method?

No samples were submitted, each collaborator being left to prepare his own. Two or three collaborators commented adversely on this plan. While the associate referee appreciates the advantages of having a number of analysts report on the results obtained by the same method on the same sample he feels that there are, on the other hand, advantages to be gained by having the method tried on a variety of preparations.

Reports were obtained from the following collaborators:

W. H. Blome, Frederick Stearns & Co., Detroit, Mich.
G. DuBois, Monsanto Chemical Works, St. Louis, Mo.
E. O. Eaton, Bureau of Chemistry, San Francisco, Calif.
J. M. Francis, Parke, Davis & Co., Detroit, Mich.

[1] *Assoc. Official Agr. Chemists, Methods*, 1920, 184.
[2] U. S. Bur. Standards, Circ. 19, 22.

H. C. Fuller, Institute of Industrial Research, Washington, D. C.

E. H. Grant, Wm. S. Merrell Co., Cincinnati, Ohio.

R. I. Grantham, Sharp & Dohme, Baltimore, Md.

B. G. Hartmann, Bureau of Chemistry, Chicago, Ill.

L. D. Havenhill and G. N. Watson, University of Kansas, Lawrence, Kans.

A. B. Lyons, Nelson, Baker & Co., Detroit, Mich.

F. A. Mallett, Standard Chemical Co., Des Moines, Iowa.

J. I. Palmore, Bureau of Chemistry, Washington, D. C.

J. Rosin, Powers-Weightman-Rosengarten Co., Philadelphia, Pa.

B. H. St. John, Wm. R. Warner & Co., New York, N. Y.

A digest of the comments received follows:

*Paragraph I, General Procedure.*

*Blome.*—Average of two determinations on 9.29% alcohol gave 9.29% when a spiral condenser was used, and 9.12% when a straight tube condenser, placed on a slant, was employed. These results were calculated from specific gravity determinations. Determined by the refractometer, the original sample contained 9.36% alcohol, the distillate obtained with the spiral condenser 9.25% and that with the straight tube condenser 9.37%. Alcohol from volumes as great as 100 cc. can safely be distilled into 50 cc. provided the distillate contains not more than 20% alcohol by volume, but greater concentrations may result in incomplete recovery. Thus when it was attempted to distil the alcohol from 200 cc. of 9.27% alcohol into 50 cc. the alcohol in the distillate corresponded to only 8.62% in the original. Placing water in the receiving flask and adding salt to raise the boiling point are unnecessary. Except in warm weather a cooling bath for the receiving flask is also unnecessary.

*Hartmann.*—Type of condenser, rate of distillation and temperature of condenser water are factors which will determine the maximum percentage of alcohol which may safely be distilled. Using a long-necked, 500 cc. Kjeldahl flask, a 15-inch Allihn condenser, condenser water at 10–12°C., and distilling 50 cc. in 10 to 20 minutes it was found that the alcohol from 100 cc. of 10% alcohol was all recovered in the first 50 cc. of distillate; in the case of 15% alcohol the results were doubtful; in the case of 20% alcohol the results were low. However, complete recovery was obtained by diluting 100 cc. of 20% alcohol to 150 cc. and distilling off 100 cc. in 20 to 25 minutes. Under these conditions 40% alcohol gave low results. The lost alcohol was not found in the distilling flask. It either remained in the condenser or was lost by volatilization from the receiving flask or both. Indications are that it remained in the condenser.

*Eaton.*—Water-alcohol mixture containing by specific gravity 9.71% alcohol by volume (by refractometer 9.72%) was distilled into one-half its volume. Alcohol calculated from specific gravity of the distillate 9.69%. (Calculated from the refractivity 9.71%.) Apparatus: 200 cc. Erlenmeyer flask, spray trap, spiral condenser, adapter, and burner guard to protect receiving flask. Thinks round-bottom flask placed with neck inclined might be better.

*Francis.*—Prefers ordinary round-bottom flask connected by spray trap to vertical spiral condenser. Alcohol from volumes as great as 100 cc. can be safely distilled into 50 cc. where the distillate contains not more than 20% of alcohol. Thus 20 cc. of alcohol diluted with 80 cc. of water and distilled into 50 cc. gave a specific gravity of 0.9520 (duplicate 0.9519), while 20 cc. of alcohol diluted with 55 cc. of water and distilled into 50 cc. gave specific gravity of 0.9520 (duplicate 0.9518). Cooling bath for the receiving flask is unnecessary if condenser is efficient and condenser water cold.

*Palmore.*—25 cc. each of water-alcohol mixtures containing 9.84% and 19.65% (by volume) of alcohol were diluted to 100 cc.; 50 cc. were distilled off. Duplicate experiments gave 9.58% and 9.66%; 19.34% and 19.40%. The addition of sodium chloride or sodium sulfate to the liquid being distilled is not of any advantage. The use of a little water in the receiving flask is unnecessary. Immersing the receiving flask in a cooling bath is unnecessary if the condensing surface is sufficient and the condenser water is cold.

*DuBois.*—50 cc. of alcohol (19.73%) diluted to 100 cc. and distilled into 50 cc. gave 19.73% (receiver dry) and 19.68% (receiver containing 2 cc. water).

*Rosin.*—If only 50 cc. is to be distilled total volume in distilling flask should not exceed 75 cc. Prefers to start with volume of preparation containing about 20 cc. alcohol, dilute to about 130 cc. and distil off 100 cc.

*Grantham.*—Prefers spiral condenser. Spray trap is advantageous. Alcohol from a volume of 100 cc. all recovered in 50 cc. distillate provided distillate contains no more than 20% alcohol.

*Mallett.*—Repeated experiments using 14.1% alcohol indicate practically no loss of alcohol. Use of a little water in the receiving flask and cooling of receiving flask both advantageous in warm weather but unnecessary at usual temperatures.

*Havenhill and Watson.*—Usual type of distilling flask objectionable because of condensation in neck above outlet. Prefers ordinary round-bottom flask with short narrow neck. If neck is inclined at an angle of 45° it serves as a spray trap. Prefers vertical spiral condenser. Has not found it satisfactory to distil 50 cc. from 100 cc. Prefers to distil 50 cc. from 75 cc. Addition of salt offers no advantage except perhaps where 100 or 125 cc. samples are to be distilled into 50 cc. No advantage in adding water to receiving flask but if room temperature is above 20°C. employs a cooling bath.

*Grant.*—The method is merely a rehash of official methods for alcohol in foods. Unnecessary verbiage should be eliminated by referring to XV, 4[1]. A 200 cc. flask is too small if there is any frothing. No advantage in distilling from a volume of 100 cc. or more into 50 cc. Necessity for finer temperature adjustments and loss of alcohol overbalance any increased accuracy obtained by the concentration. Water placed in the receiving flask is of no advantage unless the adapter dips below its surface. Lightly plugging the mouth of the receiver around the stem of the adapter with cotton to avoid air currents practically prevents loss of alcohol vapor.

*Fuller.*—No advantage in distilling from 100 cc. into 50 cc. unless alcohol content is below 5%. Prefers to use 100 cc. flask for measuring the sample and the same flask as a receiver. Cooling bath for the receiver is desirable.

*Lyons.*—Prefers to keep percentage of alcohol in the distillate below 15%, regarding 10%, on theoretical grounds, as the optimum. Addition of salt to raise the boiling point is unnecessary. Slow distillation holds back many impurities, at least in part. Refrigeration of the receiving flask is unnecessary except in very warm weather. Placing water in the receiving flask is unnecessary.

### *Paragraph II, Frothing.*

*Grantham.*—The use of tannin in liberal quantities is preferred to the proposed method.

*Havenhill and Watson.*—Dilute sulfuric acid gives satisfactory results. Tannin also is usually satisfactory.

*St. John.*—Prefers to avoid the use of a volatile acid (hydrochloric). Uses sulfuric or phosphoric in an amount equivalent to 5 cc. concentrated acid.

*Blome.*—Small quantity of tannin gives satisfactory results.

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[1] *Assoc. Official Agr. Chemists, Methods*, 1920, 173.

*Paragraph III, Charring Solids.*

*St. John.*—Dilution served to obviate the difficulty. Oil bath unnecessary.

*Grantham.*—Dilution serves. Oil bath unnecessary.

*Havenhill and Watson.*—Dilute sufficiently to keep contents of flask entirely fluid.

*Grant.*—Add more water. Do not let the flame touch the flask above the water line. Distil slowly.

*Fuller.*—Dilute considerably.

*Francis.*—Dilute.

*Mallett.*—Dilution and careful heating are sufficient precautions.

*Paragraph IV, Glycerine.*

*St. John.*—Directions are strictly correct.

*Grantham.*—Directions are correct.

*Grant.*—Fifty per cent glycerine is a little high.

*Blome.*—Keep glycerine in distilling flask below 50%.

*Paragraph V, Iodine.*

*Blome.*—Working with a solution containing 9.29% alcohol identical results were obtained by the zinc dust and thiosulfate methods, 9.25%. There is no choice between the methods so far as results are concerned but zinc dust is perhaps a little more expeditious and obviates formation of sulfur.

*Havenhill and Watson.*—Zinc dust is satisfactory.

*Grantham.*—Zinc dust is much preferred to thiosulfate. It avoids danger of formation of iodoform and of foaming as occurs almost invariably in the presence of alkali.

*Paragraph VI, Volatile Acids and Bases.*

*St. John.*—Suggests sulfuric or phosphoric acid for the neutralization of volatile bases, magnesium oxide for volatile acids.

*Grant.*—Magnesium oxide is better than sodium hydroxide, but sodium hydroxide is prescribed in the official method for wine.

*Paragraph VII, Volatile Substances.*

*St. John.*—Practically all alcoholic medicinal preparations containing volatile oils must be distilled before the shaking out with petroleum ether. Extractives prevent complete salting out of volatile oils; troublesome emulsions are formed in nearly every case. Acetone is not completely removed by this process.

*Grantham.*—The method does not result in complete removal of acetone. It is doubtful whether the separation of alcohol from acetone by the use of an immiscible solvent is practicable.

*Blome.*—The method does not completely remove acetone.

*Francis.*—The method removes only a small part of the acetone and is entirely worthless as a means of separating acetone from alcohol. Suggests as a substitute the precipitation of acetone by means of Deniges' mercuric sulfate reagent[1]. By this method a solution of 28.6% alcohol in water to which ⅓ volume of acetone was added showed 28.8% and 29.2% alcohol in duplicate determinations. The result is slightly high but is sufficiently accurate for all practical purposes.

*Paragraph VIII, Chloral Hydrate.*

*St. John.*—Surprised to see how smoothly the assay of alcohol in the presence of chloral works out by the proposed method. The point of vital importance is to use sufficient caustic soda solution to allow a considerable excess.

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[1] *J. pharm. chemie*, 1899, 9:7.

*DuBois.*—A solution containing 9.73% alcohol and 2.1 grains chloral hydrate per fluid ounce assayed 9.52% alcohol; one containing 8.18% alcohol and 91 grains chloral hydrate per fluid ounce assayed 7.57% alcohol.

*Grantham.*—To 50 cc. of a solution of 23.5% alcohol was added 10 grams of chloral hydrate and the assay made as directed, except that 25 cc. of petroleum ether was used instead of 15 cc. The specific gravity of the distillate indicated 22.5% alcohol. The distillate gave a positive reaction for chloroform. In another determination shaking out three times instead of twice with petroleum ether the alcohol was completely recovered and the test for chloroform in the distillate was negative.

*Grant.*—Chloral hydrate is so seldom used in alcoholic preparations, and it is so easy to test the distillate for chloral hydrate or chloroform that work on this method would be a waste of time. Has never met such a combination.

*Blome.*—9.29% alcohol to which chloral hydrate was added assayed 9.25%.

### *Paragraph IX, Methyl Alcohol (qualitative.)*

*Rosin.*—Has found the Deniges test for methyl alcohol to be as delicate as the author claims.

*Grantham.*—Because of the delicacy of this test would it not be advisable to have at least one confirmatory test? Few tests have received as much attention as this one; at least 50 have been published. The U. S. P. test is not very satisfactory. Uses the following: Mix 5 cc. of the sample with 5 cc. of water. Distil. To the first 1 cc. of distillate add 4 cc. of 20% sulfuric acid. Cool and add 1 gram of potassium permanganate. Allow to stand 5 minutes, filter through a small filter paper, and heat the filtrate until the pink color disappears. Mix 1 cc. with 5 cc. concentrated sulfuric acid containing 50 mgs. morphine. A pink color indicates methyl alcohol.

*Fuller.*—Finds the method a very delicate one.

*Blome.*—Followed the directions very carefully with a blank for comparison. 10% ethyl alcohol and 2% methyl alcohol gave a striking reaction almost immediately; 1%, good results in about 5 minutes; 0.5%, good results in 10 minutes; 0.2%, good results in 1 hour; 0.1%, fair results in 2 hours and good results in 3 hours. Schiff's reagent 24 hours old gave better results than the freshly prepared reagent.

*St. John.*—By the test described has been able to obtain a decidedly positive test for methyl alcohol in ethyl alcohol which there was no reason to suspect of adulteration and which by no other test would give even most faintly a positive reaction for methyl alcohol. Concludes that the test is absolutely worthless in the form given.

### *Paragraph X, Methyl Alcohol (quantitative).*

*Grant.*—The chemical method suggested is worthless.

*Blome.*—

| USED | | FOUND | |
|---|---|---|---|
| Ethyl Alcohol | Methyl Alcohol | Ethyl Alcohol | Methyl Alcohol |
| *per cent* | *per cent* | *per cent* | *per cent* |
| 10 | 0.1 | 9.98 | 0.16 |
| 10 | 0.2 | 9.55 | 0.58 |
| 10 | 1.0 | 8.50 | 2.40 |
| 10 | 2.0 | 7.34 | 4.24 |

Method gives roughly twice as much methyl alcohol as the mixture contains, and is accordingly unsatisfactory.

The following revision of the method is based on the data and criticisms received:

Introduce into a suitable flask a measured (at 20°C.) volume of the preparation not exceeding 100 cc. and containing not more than 10 cc. absolute alcohol. Add water, if necessary, to bring the total volume to 75–100 cc. Connect the flask with a suitable condenser, preferably of the spiral type, to the lower end of which is attached an adapter which extends through the neck and into the bulb of a graduated 50 cc. flask. Pack a little cotton loosely in the mouth of the flask around the stem of the adapter to prevent air currents. Protect the distilling flask so that no charring of non-volatile organic matter occurs. Distil at a moderate rate, considering the temperature of the condenser water, type and size of condenser, etc. Continue the distillation until the flask is filled nearly to the mark. If the distillate consists of a mixture of alcohol and water only, bring to a temperature of 20°C., fill to the mark with water at 20°C., mix thoroughly and determine the density at 20°C. or the refractivity at a convenient temperature, and ascertain the proportion of alcohol by volume in the distillate from the reference tables, 7 and 8. From the percentage of alcohol found in the distillate calculate the percentage in the preparation by multiplying by the proper factor.

If the preparation contains much solid matter take for distillation such a volume within the limits prescribed, that the contents of the distilling flask remain entirely fluid throughout the distillation. If the preparation contains glycerine take a volume such that the residue in the distilling flask at the end of the distillation shall contain not more than 50% glycerine; or redistil the distillate.

If the preparation contains iodine reduce with zinc dust.

If the preparation contains a volatile acid neutralize with magnesium oxide. If it contains a volatile base neutralize with dilute sulfuric or phosphoric acid. If it contains both cations of a volatile base and anions of a volatile acid distil first from an excess of magnesium oxide, then distil the distillate from an excess of sulfuric or phosphoric acid, or vice versa.

If the preparation contains camphor, chloroform, ether, or a volatile oil, dilute the portion to be distilled, if necessary, so that the alcohol content is not more than 25% by volume, saturate with common salt and shake in a separator with about 15 cc. of petroleum ether. After the liquids have separated completely draw off the lower alcoholic salt solution into a second separator and repeat the extraction with about 15 cc. of petroleum ether. Draw off the lower alcoholic salt solution into the distilling flask. Wash the two portions of petroleum ether successively with 10 cc. of saturated salt solution and add the aqueous layer to the contents of the distilling flask. Distil as above directed. If the character of the preparation is such as to render difficult or otherwise undesirable the direct shaking out, make a preliminary distillation, saturate the distillate with salt, shake out with petroleum ether, and proceed as directed.

If the preparation contains acetone add to the portion to be distilled, a sufficient amount of a solution of 5 grams of mercuric oxide in a mixture of 100 cc. of water and 20 cc. concentrated sulfuric acid to precipitate the acetone. Allow to stand until the precipitate settles, filter and distil the filtrate as directed.

If the preparation contains chloral add sufficient concentrated solution of sodium hydroxide to convert the chloral into chloroform and an excess sufficient to render the solution about 0.5N alkali. Stopper the flask and allow to stand 10 minutes. Shake out with petroleum ether and proceed as directed. If deemed advisable a preliminary distillation may be made.

If frothing due to saponin occurs add a few grams of tannin or hydrolize the glucoside by adding a little dilute sulfuric acid to the contents of the distilling flask and boiling very gently, finally increasing the heat and completing the distillation as usual.

If it is desired to test the distillate for the presence of methyl alcohol take the volume of the distillate which contains about 0.1 cc. of alcohol, dilute with water to about 4 cc. and add 1 cc. of 5% potassium permanganate solution and 0.2 cc. of concentrated sulfuric acid. After 3 minutes add a few drops of a cold saturated solution of oxalic acid. When the liquid has become colorless or pale yellow add 1 cc. of concentrated sulfuric acid, mix, and cool somewhat. To the colorless liquid add 5 cc. of Schiff's reagent (prepared by adding 100 cc. of a 0.01% solution of magenta to 2 cc. of a saturated solution of hydrogen sodium sulfite and after 5 minutes adding 2 cc. of concentrated hydrochloric acid). The appearance of a violet color after some minutes indicates the presence of methyl alcohol.

If the preparation contains methyl alcohol proceed as directed under **17** or **18** of the official methods.

Some of the collaborators commented on the official density tables for alcohol. These comments, in the opinion of the associate referee, deserve careful consideration.

St. John says that he finds it more convenient to make specific gravity determinations at room temperature, using the U. S. P. tables[1] and correction data.

Fuller dislikes the official table. In his new book[2] he has used the U. S. P. table, but states that he is sorry now that he did not use the table of Bulletin 107[3].

Lyons points out that the official tables themselves are inconsistent, the density table giving values for volume percentages of alcohol at 20°C., and the refractivity table giving the data for volume percentages of alcohol at a temperature not stated but presumably 15°C. For some concentrations the difference is not inconsiderable.

Lyons also points out that Table 7 is a *density*, not an *apparent specific gravity* table. He thinks it should be explicitly stated that in using these tables it is necessary to reduce all weighings to a vacuum basis.

There is no question but that the tables should all be on the same basis and your associate referee is of the opinion that the density table might well be replaced with an apparent specific gravity table. It is realized that in the preparation of apparent specific gravity tables it is necessary to assume standard conditions of barometric pressure, humidity and density of the weights; but errors due to slight variations in these factors are negligible for all practical purposes. Unless the table is replaced as suggested it is highly desirable that formulas for making the necessary calculations be introduced into the next revision of the Book of Methods.

## RECOMMENDATIONS.

It is recommended—

(1) That the method for alcohol as revised be studied for another

[1] U. S. Pharmacopœia, IX, 1916, 108.
[2] Chemistry and Analysis of Drugs and Medicine, 1920.
[3] U. S. Bur. Chem. Bull. 107.

year with the purpose of perfecting it and adopting it as a tentative method.

(2) That the Committee on Revision of the Methods be instructed to adjust Tables 7 and 8 to the same basis.

(3) That an apparent specific gravity table with temperature corrections be substituted for the present density table for alcohol-water mixtures; or, if the association prefers to retain the present table, that explicit directions and formulas for obtaining density from apparent weights be introduced.

## REPORT ON THE DETERMINATION OF CHLOROFORM IN DRUG PRODUCTS.

By A. G. MURRAY (Bureau of Chemistry, Washington, D. C.), *Associate Referee.*

The following method for the determination of chloroform in drug products was submitted to collaborators:

### PREPARATION OF REAGENT.

*Alcoholic sodium hydroxide.*—Dissolve 30 grams of sodium hydroxide in 30 cc. of water, cool and add sufficient methyl alcohol to make the total volume 100 cc. Allow to stand overnight and decant the supernatant solution. If the solution contains more than a trace of chloride the amount must be determined and a proper correction applied in the determination of chloroform.

### DETERMINATION.

Introduce about 10 cc. of the alcoholic sodium hydroxide solution into a 100 cc. glass-stoppered graduated flask. (Use of a funnel will prevent contact of the alkali with the upper portion of the neck of the flask and subsequent sticking of the stopper.) Add not more than 5 cc. of the sample accurately measured or weighed, containing not more than 1 gram and preferably not less than 0.1 gram of chloroform. Stopper the flask, mix the contents and allow to stand at room temperature overnight. Loosen the stopper and heat for 10 minutes on the steam bath. Cool, dilute to the mark with water and mix thoroughly. Estimate chloride in an aliquot of the solution by the volumetric method[1]. One cc. of 0.1N silver nitrate is equivalent to 3.98 mg. of chloroform.

If the sample contains chloride determine the amount and apply the proper correction; or eliminate chloride by a preliminary distillation, taking suitable precautions to prevent loss of chloroform.

A. W. Hanson of the Food and Drug Inspection Station, Chicago, Ill., reports that duplicate determinations on 5 cc. samples of a 5% solution of chloroform in alcohol gave recoveries of 99.6 and 101.6%. A 10 cc. sample of the same solution gave a recovery of 99.5%. Omitting the final heating low results were obtained (90% recovery), but on reducing

[1] *Assoc. Official Agr. Chemists, Methods*, 1920, 19.

the time of standing to 3 hours and heating for 5 minutes 99.5% recovery was obtained on a 5 cc. sample. At Hanson's suggestion the time of heating has been increased in the method as described to 10 minutes. As originally sent out the method specified 5 minutes. Hanson also suggests that the method be verified for aqueous solutions of chloroform.

W. H. Blome, of Frederick Stearns & Co., Detroit, Mich., used the method on the determination of chloroform in a sample of Syrup of White Pine Compound, in the manufacture of which 4.5 minims of chloroform per fluid ounce are used, all of which does not go into solution. The method indicated 4.8 minims per fluid ounce. Blome concludes that the method gives high results. A second determination on a sirup to which were added 3.25 minims of chloroform per fluid ounce assayed 4.3 minims, approximately 33% too high. This result is puzzling, for while a low result can be easily explained, there is no apparent explanation for a high result. Blome states, however, that it was difficult to observe the end point of the titration on account of the dark color of the solution.

M. E. Strand, of Parke, Davis & Co., reports complete recovery of chloroform in the case of a solution containing 1 gram per 100 cc., but a recovery of only 90% in the case of a solution containing 5 grams per 100 cc. By substituting potassium hydroxide for sodium hydroxide and using 30 cc. instead of 10 cc., he was able to obtain practically complete recovery of chloroform in solutions of any strength by diluting, if necessary, so that the 5 cc. used contained not more than 0.2 gram of chloroform. He states that the potassium hydroxide dissolves completely and develops less color than the sodium hydroxide solutions. He says that theoretically potassium hydroxide is a more powerful saponifying agent than sodium hydroxide.

E. O. Eaton of the Food and Drug Inspection Station, San Francisco, Calif., reports that a sample of Mallinckrodt U. S. P. chloroform assayed by the proposed method 97.2%, duplicate 97.5%. Lower results were obtained if the final heating was omitted. Low results were obtained if the solutions were allowed to stand only 4 hours instead of overnight.

These results indicate the need for further directions. While two of the collaborators report satisfactory results two others report quite unsatisfactory results, one of these finding that the recovery is not complete and the other obtaining results indicating about one-third more chloroform than is actually present. Further work to determine just what conditions influence the reaction is necessary. Strand's suggestion that potassium hydroxide be substituted for sodium hydroxide should be considered. A possible explanation of the better results which he obtained by the modifications suggested is the increased quantity of reagent used. Sodium and potassium hydroxides are about equally strong

bases, molecule for molecule. Since potassium hydroxide usually contains considerably more moisture than does sodium hydroxide and since the molecular weight of potassium hydroxide is 40% greater than that of sodium hydroxide, it is evident that, weight for weight, sodium hydroxide yields a much stronger solution of alkali than does potassium hydroxide. The greater solubility of potassium hydroxide may, however, warrant its substitution for sodium hydroxide.

RECOMMENDATION.

It is recommended that the method for the determination of chloroform in drug products as outlined in this report be studied another year, with special reference to the criticisms that have been offered by the collaborators.

## REPORT ON THE DETERMINATION OF CHLORAL HYDRATE IN DRUG PRODUCTS.

By A. G. MURRAY (Bureau of Chemistry, Washington, D. C.), *Associate Referee.*

The following method for the determination of chloral hydrate in drug products was submitted to collaborators:

Proceed as directed for chloroform. (See report on the determination of chloroform, page 530). One cc. of 0.1N silver nitrate is equivalent to 5.51 mg. of chloral hydrate.

If the sample contains chloride the amount must be determined and the proper correction applied, or a preliminary distillation may be made. For the latter purpose a Hortvet tube[1] may be used. Distil with steam until the distillate amounts to three times the volume of the sample taken.

In addition collaborators were requested to report on the United States Pharmacopœia method[2] for the assay of chloral hydrate.

E. O. Eaton reports that a sample of Mallinckrodt's U. S. P. chloral hydrate assayed by the proposed method 98.4%, a duplicate determination giving the same result. Using 10 cc. of alcohol containing 2 grams of chloral hydrate, complete recovery was not obtained by distilling with steam until the distillate amounted to 30 cc. A similar failure resulted when 10 cc. of an aqueous 40% solution was similarly distilled. The United States Pharmacopœia method gave slightly higher than 100%. The method is faulty and could not be trusted in the case of an unknown mixture.

W. H. Blome, on a sample of bromo-chloral compound, containing 120 grains of chloral hydrate per fluid ounce, found, after making cor-

[1] *Assoc. Official Agr. Chemists, Methods*, 1920, 177.
[2] U. S. Pharmacopœia, IX, 1916, 108.

rection for bromide, 130 grains of chloral hydrate per fluid ounce, an excess of about 8%.

A. W. Hanson worked on an alcoholic solution of chloral hydrate—5 grams per 100 cc. Triplicate determinations on 5 cc. portions (0.25 gram chloral hydrate) gave 0.248, 0.248, and 0.242 gram. By following strictly the United States Pharmacopœia directions, on 4-gram samples the recovery was 4.00 and 4.03 grams. Increasing the amount of alkali used from 30 to 50 cc. 4 grams assayed 102%. By increasing the volume of alkali to 60 cc. and the time of standing from 2 minutes to 1 hour the recovery indicated was 120%, due to hydrolysis of chloroform.

RECOMMENDATION.

The correspondence in connection with this investigation has developed the fact that the use of chloral hydrate in medicine has almost reached the vanishing point. It appears undesirable to take up further space for a method for which there will probably be so little need. It is recommended that no further work be done upon this subject.

## INVESTIGATION OF ANALYTICAL METHODS FOR THE ANALYSIS OF SILVER PROTEINATE.

By W. L. MITCHELL[1] (U. S. Food and Drug Inspection Station, New York, N. Y.), *Associate Referee.*

In "New and Nonofficial Remedies, 1921", several methods are outlined for the determination of silver in silver proteinate and organic silver compounds. Under the preparation "Cargentos" the following method is outlined:

(1) From 0.6 to 1 gram cargentos is weighed into a crucible and ignited gently at first, afterward with full flame and a Bunsen burner, until the ash is light in color. The residue is treated with concentrated nitric acid, and, if completely dissolved, the solution is diluted with 50 cc. water, 2 cc. ferric ammonium sulfate solution added and directly titrated with 0.1N potassium thiocyanate. If after treating with nitric acid an insoluble residue of silver chloride remains, it is collected on a filter, washed with water, dried, ignited and weighed as silver chloride; the silver content of the filtrate is determined by titration with 0.1N thiocyanate as before, and the silver so found is added to that obtained in the silver chloride.

Under the preparation "Solargentum-Squibb", another method is outlined as follows:

(2) To about 1 gram of powdered solargentum-Squibb, accurately weighed into a porcelain crucible, add a mixture of 4.5 gram of lead oxide and 0.5 gram of powdered tartaric acid. Rotate and mix in a crucible. Heat cautiously until thoroughly car-

[1] Presented by G. H. Arner

bonized and then heat in a blast flame until the lead button formed is about half its original size. Allow the crucible to cool, then place it in a beaker and dissolve the lead button containing the silver in dilute nitric acid. Transfer the liquid, with washings, into an Erlenmeyer flask and titrate the silver nitrate with 0.1N potassium thiocyanate volumetric solution, using ferric ammonium sulfate as indicator. The silver content corresponds to not less than 19 per cent and not more than 23 per cent of metallic silver (each cc. of 0.1N potassium thiocyanate volumetric solution is equivalent to 0.0107 gram silver).

A modification of a method outlined under "Protargentum-Squibb" was used except that the use of potassium permanganate was omitted:

(3) To about 1 gram silvol add 20 cc. concentrated sulfuric acid. Heat over a free flame until solution is practically colorless and about 10 cc. of the solution remain in the flask. Cool the residue with water and dilute to 300 cc. Add 5 cc. of concentrated nitric acid and 5 cc. of ferric ammonium sulfate solution T. S. and titrate with 0.1N potassium thiocyanate.

Nitrogen was determined using the Gunning method[1].

The results of analysis are listed in the following table:

| | SILVER DETERMINATIONS | | | NITROGEN DETERMINATIONS |
|---|---|---|---|---|
| | Method 1 | Method 2 | Method 3 | Gunning Method |
| | *per cent* | *per cent* | *per cent* | *per cent* |
| Argyrol | 18.28 | 18.39 | ..... | 7.44 |
| Protargal | 8.17 | ..... | ..... | 13.94 |
| | 7.85 | ..... | ..... | ..... |
| Silvol | 19.20 | 19.67 | 20.49 | 11.56 |

## COMMENTS ON METHODS.

*Method 1.*—There is no difficulty in carrying out this method if the product is free from chlorides. In case chlorides are present it becomes rather difficult to separate the insoluble silver chloride from the acid-insoluble ash.

*Method 2.*—The material after fusion is a brown glossy mass which is difficultly soluble in acid. Here again in case chlorides are present trouble arises in separating the insoluble silver salt.

*Method 3.*—This method is the least difficult to carry out and apparently gives correct results. Any insoluble silver salts are converted into the soluble silver sulfate.

## RECOMMENDATIONS.

It is recommended—

(1) That as silver proteinate generally contains chlorides in small amounts, no further work be done on Methods 1 and 2.

(2) That a further collaborative study be made of Method 3.

[1] *Assoc. Offic. Agr. Chemists, Methods*, 1920, 7.

# REPORT ON THE DETERMINATION OF CAMPHOR IN PILLS AND TABLETS BY THE ALCOHOL DISTILLATION METHOD.

By GAIL H. ARNER (U. S. Appraiser's Stores, New York, N. Y.), *Associate Referee.*

No one method of the several proposed for the estimation of camphor is in general use or gives entirely accurate results. H. C. Fuller[1] and E. K. Nelson[2] proposed to determine camphor by taking advantage of the fact that it forms a well-defined oxime. In a letter to the Referee on Drugs, Nelson states that the oxime method has proved unreliable, both in cooperative work done in the United States and in work done in other countries. The determination of camphor by loss due to evaporation is uncertain, owing to the possible presence of other volatile substances.

The polariscopic method seems to be used most widely and to give the best results. In the method reported by Edwin Dowzard[3] the camphor is steam-distilled in a special apparatus, extracted with benzol and the rotation of the benzol solution taken. Arthur T. Collins[4] states that the determination of camphor in alcohol by the polariscope gives very accurate results when a control is used.

It is proposed to determine collaboratively the camphor in pills and tablets by two promising methods. One of these methods is described in the ninth revision of the United States Pharmacopœia[5], and the other has been outlined by Nelson[6]. The latter method involves steam distillation and the use of chloroform in the steam generator to prevent blocking in the condenser tube. The camphor is extracted from the distillate, made up to a definite volume and polarized.

It is necessary to run a control with each determination, using a sublimed sample of the camphor under examination as different lots of natural camphor show variations in rotation.

It is proposed to study these two methods collaboratively.

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[1] U. S. Bur. Chem. Circ. 77: 1911.
[2] U. S. Bur. Chem. Bull. 162: (1912), 208.
[3] *J. Ind. Eng. Chem.*, 1914, 6: 489.
[4] *Ibid.*, 1912, 4: 514.
[5] U. S. Pharmacopœia, IX, 1916, 233.
[6] Personal communication.

# ESTIMATION OF SANTALOL IN SANTAL OIL BY THE ASSAY METHOD OF THE UNITED STATES PHARMACOPŒIA AND BY THE DISTILLATION METHOD

By C. W. HARRISON (U. S. Food and Drug Inspection Station, Park Avenue Building, Baltimore, Md.), *Associate Referee.*

The collaborative work on the United States Pharmacopœia[1] and distillation[2] methods for the determination of santalol in santal oil, begun in 1920, was continued. The same general plan of work was followed, except that the instructions sent to collaborators were more specific, it having appeared from the previous year's results that the collaborators did not entirely understand the procedure in either method.

Four samples were sent out with detailed instructions as follows:

Place about 21 cc. of oil in an acetylization flask with an equal volume of acetic anhydride and a few grams of fused sodium acetate and boil for about an hour. Transfer contents of flask to a small separatory funnel and wash four times with 20 cc. of sodium carbonate solution (5 grams in 100 cc.). (Test the final wash solution with phenolphthalein to be certain that it is alkaline, indicating that all free acid has been removed. If the wash solution is not alkaline to phenolphthalein, continue washing with sodium carbonate solution until the washings show an alkaline reaction.)

Transfer the acetylated oil to a 25 cc. cylinder, fill nearly to the surface of the oil with granular (4-mesh) anhydrous calcium chloride and allow the mixture to stand overnight. (This is necessary to dry the oil completely.) Pass through a dry filter.

Into a dry, tared, 100 cc. Erlenmeyer flask run about 5 cc. of the dry, filtered, acetylated oil and weigh accurately. Then add 50 cc. of approximately 0.5N alcoholic potash solution. Measure into another flask 50 cc. of the alcoholic potash solution, using the same pipet, drain the same length of time, and carry through as a blank.

Heat on the steam bath about 30 minutes, using a small funnel as a reflux, cool and titrate with 0.5N acid and phenolphthalein, titrating the blank at the same time. Subtract the number of cubic centimeters of 0.5N acid required for the titration of the sample from the number required by the blank and calculate the percentage of santalol from the formula:

$\frac{A \times 11.11}{B-(A \times 0.021)}$, "A" representing the difference in cc. between the sample and blank, and "B" the weight of acetylated oil used.

To the flask containing the residue after titration, add 1 or 2 drops of alcoholic potash to render it faintly alkaline, place on the steam bath and evaporate to a small volume (about 10 cc.). (This is easily and quickly accomplished by passing a tube attached to a vacuum into the neck of the flask nearly to the surface of the liquid, thus carrying off the hydroalcoholic vapors as fast as they form.)

Transfer the residue in the flask, by aid of a small funnel, to the distillation apparatus, carefully washing the adhering material from the flask and funnel into the apparatus with 15 to 20 cc. of approximately 5% acid. (Either hydrochloric or sulfuric acid is satisfactory, but it is necessary to use the same kind of acid as was used for the previous titration.) Add also a drop or two of methyl orange TS to be certain of an excess of mineral acid.

[1] U. S. Pharmacopœia, IX, 1916, 296.
[2] *J. Assoc. Official Agr. Chemists, Methods*, 1920, 425.

Steam-distil the material and titrate the volatile acids with 0.5N alkali and phenolphthalein. Most of the acids come over in the first 250 cc. of distillate, but the distillation should be continued until 20 cc. of distillate requires only 1 or 2 drops of 0.5N alkali to neutralize it. Calculate the santalol content from the formula:

$$\frac{\text{Cc. of 0.5N alkali} \times 11.11}{\text{Weight of oil} - \text{cc. of 0.5N alkali} \times 0.021}.$$

Results were received from one collaborator only, and these did not show a satisfactory agreement with the results obtained by the associate referee, the disagreement being about equally great in the case of both methods.

The four samples consisted of two pure santal oils, designated as Nos. 1 and 3, and two compound oils, designated as Nos. 2 and 4, respectively.

Compound No. 2 was prepared by adulterating Santal Oil No. 1 with 12% cocoanut oil and 5% cubeb oil, and Compound No. 4, by adulterating Santal Oil No. 3 with 18% cocoanut oil and 10% cubeb oil.

The following results, expressed as per cent, were obtained:

*Results by the U. S. P. and distillation methods*.*

| ANALYST | SAMPLE 1 | | SAMPLE 2 | | SAMPLE 3 | | SAMPLE 4 | |
|---|---|---|---|---|---|---|---|---|
| | A | B | A | B | A | B | A | B |
| A. W. Hanson, U. S. Food and Drug Inspection Station, Chicago, Ill. | 91.4<br>91.5 | 91.2<br>92.0 | 89.3<br>90.3 | 81.2<br>79.4 | 92.3<br>91.1 | 92.3<br>91.2 | 86.8<br>89.0 | 71.9<br>69.1 |
| C. W. Harrison..... | 87.0<br>87.7 | 86.4<br>86.5 | ....<br>87.4 | ....<br>72.4 | ....<br>88.0 | ....<br>87.7 | ....<br>86.4 | ....<br>68.1 |

*"A" refers to U. S. P. method; "B" refers to distillation method.

## COMMENTS.

This table shows the lack of agreement between the analysts when reporting results by either method. The inference, therefore, is that the fault lies in the present procedure of acetylating the oil since this step is the same in both methods. The acetylization procedure should be changed so that the results will show invariably a uniform saponification number.

The distillation method, while not entirely satisfactory, shows the adulteration much better than the United States Pharmacopœia method. This is illustrated by Hanson's results, about which he makes the comment that the distillation method indicates that Samples 2 and 4 are adulterated, although they might pass by the United States Pharmacopœia method.

Since these two samples were adulterated with 17 and 28 per cent, respectively, of foreign oils, the weakness of the United States Pharmacopœia method, when dealing with adulterated oils, is at once apparent.

It has been pointed out by E. K. Nelson[1] that the distillation method possesses certain advantages over the United States Pharmacopœia method when working with pure oil, it being his opinion that it would give more nearly the true percentage of santalol present.

It may be concluded, therefore, that since the distillation method possesses certain advantages over the United States Pharmacopœia method and gives more accurate results on both pure and adulterated oils, it warrants further study. If the procedure of acetylating the oil can be satisfactorily solved, the method will be suitable for presentation to the association as a provisional method.

## REPORT ON TURPENTINE.

By J. O. CLARKE ( U. S. Food and Drug Inspection Station, Savannah, Ga.), *Associate Referee.*

The present tentative method for the detection of mineral oils in turpentine and a method proposed by A. E. Paul[2] were studied. Polymerization is effected in the Paul method with sulfuric acid, followed by fuming nitric acid. The accuracy of the usual methods for specific gravity and refractive index was also studied, since these items could easily be handled on the same samples used for the polymerization work.

Collaborators were requested to make the following determinations on three samples of turpentine: Specific gravity and refractive index, using the official methods[3]; polymerization by the fuming sulfuric acid method, using the official methods; and polymerization by the sulfuric nitric acid method, using the Paul method.

The samples were designated as A, B and C. Sample A was an authentic specimen of pure gum turpentine, redistilled in the laboratory. Samples B and C were portions of A, containing, respectively, 5 per cent and 10 per cent of kerosene.

The detailed results obtained by the collaborators on the methods for mineral oils are given in Table 1.

### COMMENTS BY COLLABORATORS.

The following brief of the comments by the collaborators should be considered in studying the tables:

*J. M. Anderson.*—The sulfuric-nitric acid method is impracticable, owing to the amount of time consumed; danger of overheating in adding turpentine distillate, causing loss of low boiling adulterant; inability to check results on repeated trials; and difficulty in removing residue (when of a heavy consistency) left in still on first steam distillation.

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[1] *J. Assoc. Official Agr. Chemists*, 1921, 5: 169.
[2] *J. Ind. Eng. Chem.*, 1909, 1: 27.
[3] *Assoc. Official Agr. Chemists, Methods*, 1920, 306.

TABLE 1.

*Collaborative study of polymerization methods on turpentine.*

| COLLABORATORS | SAMPLE A (PURE TURPENTINE) | | | | SAMPLE B (5% KEROSENE) | | | | SAMPLE C (10% KEROSENE) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Residue Fuming Sulfuric Acid Method. | Refractive Index of Residue 20°C. | Residue Sulfuric-Nitric Acid Method. | Refractive Index of Residue 20°C. | Residue Fuming Sulfuric Acid Method. | Refractive Index of Residue 20°C. | Residue Sulfuric Nitric Acid Method. | Refractive Index of Residue 20°C. | Residue Fuming Sulfuric Acid Method. | Refractive Index of Residue 20°C. | Residue Sulfuric-Nitric Acid Method. | Refractive Index of Residue 20°C. |
| | *per cent* | | *per cent* | | *per cent* | | *per cent* | | *per cent* | | *per cent* | |
| J. M. Anderson, United Drug Co., Boston, Mass. | 2.20<br>2.08 | 1.5036<br>1.5042 | 0.20<br>0.60 | 1.4502<br>1.4475 | 5.20<br>5.00 | 1.4651<br>1.4646 | 3.95<br>4.45 | 1.4384<br>1.4405 | 10.60<br>10.68 | 1.4603<br>1.4595 | 7.08<br>7.30 | 1.4358<br>1.4358 |
| W. C. Smith, Bureau of Chemistry, Washington, D. C. | 0.4 | ... | 0.2 | ... | 3.40 | 1.4510 | 3.30 | 1.4400 | 6.50 | 1.4426 | 6.10 | 1.4370 |
| C. K. Glycart, U. S. Food and Drug Inspection Station, Transportation Building, Chicago, Ill. | 0.96<br>1.12 | ...<br>... | 0.60<br>0.50 | ...<br>... | 5.60<br>5.60 | 1.4670<br>... | 5.70<br>5.50 | 1.4600<br>... | 9.60<br>8.80 | 1.456<br>... | 9.40<br>10.30 | 1.4500<br>... |
| J. O. Clarke. | 0.80<br>0.80 | 1.4888<br>... | ...<br>None | ...<br>... | 5.60<br>5.60 | 1.4515<br>1.4540 | 3.50<br>3.70 | 1.4330<br>1.4340 | 10.00<br>10.40 | 1.4460<br>1.4458 | 6.80<br>7.20 | 1.4365<br>1.4320 |
| C. W. Harrison, U. S. Food and Drug Inspection Station, Park Avenue Building, Baltimore, Md. | 1.1<br>1.1 | ... | None | ... | 3.6<br>4.8<br>3.2<br>3.6 | 1.4517 | 2.3<br>2.3 | 1.4338 | 7.2<br>7.6 | 1.4452 | 5.3<br>5.3 | 1.4340 |
| Maximum | 2.20 | 1.5042 | 0.60 | 1.4502 | 5.60 | 1.4670 | 5.70 | 1.4600 | 10.68 | 1.4603 | 10.30 | 1.4500 |
| Minimum | 0.40 | 1.4880 | None | 1.4475 | 3.20 | 1.4510 | 2.30 | 1.4330 | 6.50 | 1.4426 | 5.30 | 1 4320 |

*W. C. Smith.*—The accuracy of the two methods is the same. The fuming sulfuric acid method is preferable because it can be run in a much shorter time and on a smaller sample.

*C. K. Glycart.*—The fuming sulfuric acid method appears to give satisfactory results, but much difficulty was experienced in the preparation of the 38N sulfuric acid reagent. The sulfuric-nitric method appears to give satisfactory results, but requires much care in its manipulation. In the examination of an unknown sample, the analyst would feel more certain of the results than by the more empirical fuming sulfuric acid method.

It would seem that the sulfuric-nitric acid method should be seriously considered as an alternative.

*C. W. Harrison.*—Sample A is apparently a pure turpentine, even though it leaves a residue when treated by the official method equivalent to 1.1% by volume. It seems that in computing the amount of mineral oil in samples B and C, this figure should be subtracted from the percentage of residue found. Sample A, when treated by the sulfuric-nitric acid method, yields no residue, while samples B and C show 2.3 and 5.3% residue, respectively, and apparently there should be no deduction from these figures, the inference being that they represent the volume percentage of mineral oil present in the samples as determined by this method.

The refractive indices of the residues obtained by the sulfuric-nitric acid method are lower than those obtained by the fuming sulfuric acid method, indicating that the sulfuric-nitric acid method gives a residue containing less of the turpentine polymerization products. The residues from Samples B and C obtained by the sulfuric-nitric acid method, judging by the refractive index, are of a more uniform composition and nearer the range for gasoline than the results obtained on these samples by the fuming sulfuric acid method. I am, therefore, inclined to believe that the sulfuric-nitric acid method gives results which more nearly show the true percentage of mineral oil present.

I rather favor the sulfuric-nitric acid method as it is less trouble and does not require the standardization of the acid. Pure turpentine does not show an unpolymerized residue when treated by this method.

## *Fuming Sulfuric Acid Method.*

This method gave concordant results, and, with one exception, the recovery of the adulterant was remarkably good. With pure gum turpentine a residue of about one per cent is expected. If more than this is found, the refractive index of the residue should indicate whether or not a petroleum product is present.

The criticism most frequently offered was the difficulty in preparing the sulfuric acid reagent. Some work was done on this point by the writer, and the difficulty disappeared after a little experience. If the concentrated sulfuric acid used is boiled for several hours, it finally reaches a concentration of about 97 per cent sulfuric acid, and the preparation of acid of this strength effects considerable saving in the quantity of fuming acid required. One of the collaborators stated that the method of converting the sulfuric acid into ammonium sulfate and weighing was not so satisfactory as determining the strength by titration. This has been the experience of the writer. More work can profitably be done on the preparation of the reagent.

The time of the initial reaction affected the recovery of the mineral oil. A slow addition of the sample appeared to lower the results; on

the other hand, when the turpentine was added to the acid too rapidly, too much heat was developed, with possible loss of a part of the sample. In studying this point, six duplicate determinations were made with strict adherence to the method in all respects except that the time consumed in adding the sample was varied. In all determinations the acid was cooled to about 5°C. in ice water, the sample added under accurate time control and the temperature taken at the moment the last drop of turpentine was added. Sample C, containing 10 per cent of mineral oil, was used. A study of Table 2 shows that about half a minute should be consumed in adding the sample.

TABLE 2.
*Influence of time of addition of samples on results.*

| TIME | TEMPERATURE | | RESIDUE |
|---|---|---|---|
| | Beginning | End | |
| *Minutes* | °C. | °C. | *per cent* |
| 0.5 | 5.0 | 67 | 9.2 |
| 1.0 | 5.0 | 55 | 8.8 |
| 2.0 | 5.0 | 39 | 8.8 |
| 3.0 | 5.0 | .. | 8.0 |
| 4.0 | 5.0 | 23 | 8.0 |
| 5.0 | 5.0 | .. | 7.6 |

*Sulfuric-Nitric Acid Method.*

The results by this method in general were low. One of the collaborators recovered practically all of the adulterant in Samples B and C. However, the average recovery was not so satisfactory. The method appears to have some merit and might be useful as an alternative.

The first distillation should be continued until 400 cc. of the distillate are obtained. An experiment by the writer, using Sample C which contained 10 per cent of kerosene and measuring the oil recovered in each 50 cc. fractions, gave the following figures:

| | OIL | | OIL |
|---|---|---|---|
| | *cc.* | | *cc.* |
| 1st fraction | 25.0 | 5th fraction | 8.5 |
| 2nd fraction | 24.0 | 6th fraction | 3.5 |
| 3rd fraction | 20.0 | 7th fraction | 1.5 |
| 4th fraction | 15.0 | 8th fraction | 1.0 |

Not all of the oil was recovered in the first 100 cc. of the second distillate. Using Sample A (pure turpentine) 10.0 cc. of oil were recovered in 400 cc. of the distillate, and when the distillation was continued 2.0 cc. more were recovered in the next 400 cc. With Sample C, containing 10 per cent of kerosene, 11.5 cc. of oil were recovered in the

first 100 cc. of distillate, giving 4.2 cc. residue after polymerization with fuming nitric acid. On continued distillation to a total volume of 900 cc. 8.0 cc. more oil were recovered, which gave 2.6 cc. residue on final polymerization. The second distillation should be continued until the total distillate measures about 900 cc.

TABLE 3.

*Collaborative study of specific gravity and refractive index on turpentine.*

| COLLABORATOR | SAMPLE A | | SAMPLE B | | SAMPLE C | |
|---|---|---|---|---|---|---|
| | Refractive Index 20°C. | Specific Gravity 20°/4°C. | Refractive Index 20°C. | Specific Gravity 20°/4°C. | Refractive Index 20°C. | Specific Gravity 20°/4°C. |
| J. M. Anderson. | 1.4712<br>..... | 0.86271<br>0.86278 | 1.4703<br>..... | 0.86040<br>0.86036 | 1.4683<br>..... | 0.85670<br>0.85663 |
| W. C. Smith. | 1.4720 | 0.86396 | 1.4708 | 0.86137 | 1.4692 | 0.85824 |
| C. K. Glycart. | 1.4757 | 0.8639 | 1.4720 | 0.8597 | 1.4695 | 0.8571 |
| L. A. Salinger* | 1.4719<br>1.4720 | 0.86410<br>0.86400 | 1.4703<br>1.4704 | 0.8603<br>0.8600 | 1.4695<br>1.4694 | 0.8574<br>0.8575 |
| J. O. Clarke. | 1.4724<br>.. . | 0.86360<br>0.86369 | 1.4711<br>.... | 0.86063<br>0.86060 | 1.4697<br>..... | 0.85780<br>0.85780 |
| C. W. Harrison. | .. . | 0.86432 | ..... | 0.86104 | ... . | 0.85779 |
| Maximum.. .... | 1.4757 | 0.86432 | 1.4720 | 0.86137 | 1.4697 | 0.85824 |
| Minimum.. .... | 1.4712 | 0.86271 | 1.4703 | 0.85970 | 1.4683 | 0.85663 |

*U. S. Food and Drug Inspection Station, Savannah, Ga.

The results of these determinations were surprising. A study of Table 3 shows quite a discrepancy among different operators, although the duplicate determinations by the same operator check very closely. Probably the variation in refractive indices is due to inaccurate refractometers rather than to faulty manipulation. The determinations on specific gravity show a rather wide range, differing materially in the third place.

## RECOMMENDATIONS.

It is recommended—

(1) That the fuming sulfuric acid method be further studied with

especial attention to the preparation of the reagent and that the following form be used:

*I. Polymerization—Fuming Sulfuric Acid Method.*

REAGENT.

*38N sulfuric acid.*—Mix 140 grams of concentrated sulfuric acid with sufficient liquid, fuming sulfuric acid (about 100 grams), to obtain an acid slightly stronger than 38N. Determine the exact strength of this mixture and also of the concentrated acid as follows: Weigh out 6-8 grams in a bulb, having a capillary tube in the lower end and a tube with a stop-cock in the upper end, fitted with a platinum wire for suspending on a balance. (The bulb is filled by the aid of a slight vacuum; the lower end of the capillary is emptied by closing the stop-cock simultaneously with the withdrawal of the capillary from the acid; and the tip of the bulb is then wiped off first with a wet and then with a dry piece of cloth.) Run the acid into cold water, make up to volume and titrate an aliquot of the solution against standard alkali. Calculate the sulfur trioxide content of the acid and add sufficient concentrated sulfuric acid to make it exactly 82.38% of $SO_3$. (The acid must be carefully protected against absorption of water from the air.)

DETERMINATION.

Place 20 cc. of the 38N sulfuric acid (82.38% $SO_3$) in a graduated narrow-necked Babcock flask, stopper, place in ice water and cool. Add 5 cc. of the turpentine at such a rate that it is all added in 30 seconds, meanwhile revolving the flask in the ice water, so that the sample is mixed with the acid as added. When the mixture no longer warms on shaking, agitate thoroughly, place in a water bath and heat to 60°–65°C. for about 10 minutes, keeping the contents of the flask thoroughly mixed by vigorous shaking 5 or 6 times. Cool to room temperature and fill the flask with concentrated sulfuric acid until the unpolymerized oil rises into the graduated neck. Centrifuge 4–5 minutes at about 1200 revolutions per minute, or allow to stand for 12 hours. Read the unpolymerized residue, notice its consistency and color and determine the refractive index.

(2) That the sulfuric-nitric acid method be further studied using the following slightly modified form:

*II. Polymerization.—Sulfuric-Nitric Acid Method.*

REAGENTS.

(a) *Concentrated sulfuric acid.*—Specific gravity, 1.84.
(b) *Fuming nitric acid.*—Specific gravity, 1.50.
(c) *Concentrated nitric acid.*—Specific gravity, 1.42.

DETERMINATION.

Place 100 cc. of the sample in a 500 cc. Kjeldahl flask and distil in a current of live steam until 400 cc. of distillate are collected. Transfer the distillate and residue to separatory funnels and tap the water off. Return the separated oil from the distillate to the Kjeldahl flask, cool in ice water and treat with 50 cc. of concentrated sulfuric acid. (The acid should be added slowly and the mixture constantly agitated.) When the reaction is complete, cool thoroughly and add 25 cc. of water. Distil the polymerized mixture in a current of live steam, collecting 900 cc. of distillate. Add the separated oil from this distillate to the residue from the first distillation.

Place a volume of fuming nitric acid, equal to three times the volume of the combined oils, in a separatory funnel and cool thoroughly in ice water. Add the com-

bined oils, drop by drop, shaking carefully and keeping the mixture cool. After all the oil has been added, allow to react a few moments and draw off the acid layer. Wash the remaining oil once with fuming nitric acid, twice with strong nitric acid and finally several times with water. Measure the volume and determine the refractive index.

(3) That the method of Grotlisch and Smith[1] for the determination of coal tar oils in turpentine be studied.

## VOLUME WEIGHT DETERMINATIONS OF CRUDE DRUGS AND SPICES.

By ARNO VIEHOEVER (Bureau of Chemistry, Washington, D. C.), *Associate Referee.*

The subject of volume weight and its importance in drug inspection has been discussed in previous reports[2] of the Associate Referee on Medicinal Plants.

The work for 1921 followed the two following distinct lines:

### *Method I.*

This method, determining the weight of a certain volume, is practically the same as that adopted in grain standardization[3], except that it is more simple in that the special Boerner apparatus is not used.

It consists in filling a graduated cylinder up to the mark with the material of questionable quality, determining its weight and repeating the same procedure with a sample of the drug of good quality.

### *Method II.*

This method determines the apparent, or absolute specific weight of substances in the Kunz-Krause apparatus[4]. The slightly modified apparatus used to obtain the data recorded in the table consists of two parts, one fitting, with a ground joint, into the other. (See Fig. 2.) The capacity of 100 cc., to the mark in the neck, was found to be a suitable size. In order to ascertain the usefulness of the apparatus it was submitted to collaborators, together with test material and the following instructions:

(1) Standardize pycnometer by filling it from a buret with water at a known temperature (approximately 20°C.) to a convenient point in the stem and marking this point. The reading on the buret will be the volume of the pycnometer.

(2) Take a given weight of a chosen drug (say 20 grams) and transfer to the empty pycnometer from which the cover has been removed.

(3) The liquid considered suitable for filling the pycnometer is kerosene which has been dehydrated with anhydrous sodium sulfate. Replace the cover and fill the pycnom-

[1] *J. Ind. Eng. Chem.*, 1921, 13: 791.
[2] *J. Assoc. Official Agr. Chemists*, 1920, 4: 154; 1921, 4: 412.
[3] U. S. Dept. Agr. Bull. 472.
[4] *Ber. pharm. Ges.*, 1919, 2: 150.

eter at the same temperature, from a buret containing the kerosene, through the neck of the pycnometer to the mark in the stem. Where the specific gravity of powders is being determined it might be better to introduce a given volume (say 10 cc.) of the liquid into the pycnometer before introducing the drug. The difference between the volume of the pycnometer and the amount of the liquid used will be the volume of the material.

$$\text{Then specific gravity of the material} = \frac{\text{weight of material}}{\text{volume of material}}.$$

Materials suggested for the test are fruits such as caraway, fennel, oval and ordinary coriander; seed such as mustard seed; various powdered vegetable drugs such as pepper, hydrastis and senna; spore drugs, such as *Lycopodium;* and other drugs, such as *Lupulin* and *Kamala.*

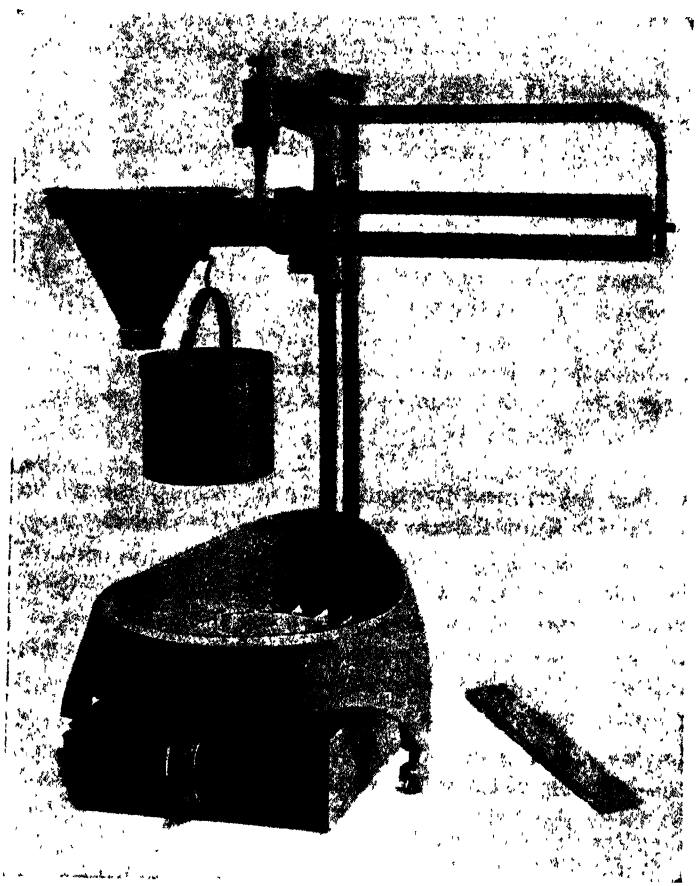
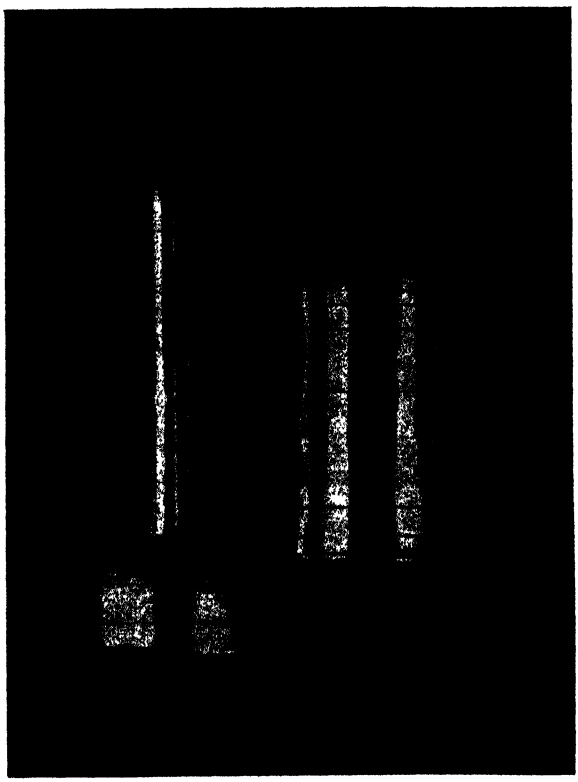


FIG. 1—BOERNER'S SPECIAL APPARATUS FOR TESTING WEIGHT PER BUSHEL. THE HOPPER IS SWUNG TO THE LEFT. THE FILLED TEST KETTLE BALANCES THE SPECIAL BEAM.

The results of determinations are believed to be useful in suggesting the presence of foreign material such as dirt and sand; in indicating the quality of the drug, such as

immature or inferior drugs and partially or wholly extracted plant products; and perhaps in indicating also the species of closely related seed, as in the case of mustards and other Brassica seeds grown mainly for their fixed-oil content.



APPARATUS FOR VOLUME WEIGHT DETERMINATION.

FIG. 2.—THE PARTS SHOWN ARE FITTED TOGETHER WITH A GROUND JOINT. × ½.

## DISCUSSION OF RESULTS.

Analytical data are available on both methods, but are especially numerous on Method I. (See former reports.) From a critical study of the data on Method I, it is apparent that considerable variation exists, even in material of practically the same quality. It seems evident that the manner of filling the cylinder with the dry substances enters materially into consideration, and explains the varying and unsatisfactory results. Similar observations had been made in the work on grain standardization and led to the devising of special apparatus, which determines the rate of flow and amount of material put into the measure. (See Fig. 1).

TABLE 1.

*Volume weight determinations.*

| PRODUCT | CONDITION | E. O. EATON* | C. K. GLYCART† | R. G. CAPEN‡ | C. S. BRINTON¶ |
|---|---|---|---|---|---|
| Acheen pepper | Whole | .... | .... | 0.85 | .... |
| Acheen pepper | Ground | .... | 1.42 | 1.36 | 1.33 |
| Aleppey pepper | Whole | .... | .... | 1.13 | .... |
| Aleppey pepper | Ground | 1.33 | 1.27 | 1.36 | 1.33 |
| Yellow mustard | Whole | .... | .... | .... | 1.19–1.20 |
| White mustard | Whole | .... | .... | 1.20 | .... |
| California Trieste | Whole | .... | .... | 1.15 | .... |
| Japanese mustard | Whole | .... | .... | 1.12 | .... |
| Chinese mustard | Whole | 1.20<br>1.143**<br>1.136**<br>1.162** | 1.14 | 1.16 | 1.19 |
| Chinese colza | Whole | 1.162 | 1.17 | 1.16 | 1.14 |
| Coffee, green, (short berry) | Whole | .... | .... | .... | 1.22 |
| Coffee, green, Mocha | Whole | .... | .... | .... | 1.21 |
| Caraway seed | Whole | .... | .... | .... | 1.18 1.13<br>1.12 1.16 |
| Coriander | Whole | .... | .... | .... | 0.60 |
| Poppy seed (blue) | Whole | .... | .... | . . . | 1.10—1.15 |

*U. S. Food and Drug Inspection Station, San Francisco, Calif.
†U. S. Food and Drug Inspection Station, Chicago, Ill.
‡Bureau of Chemistry, Washington, D. C.
¶U. S. Food and Drug Inspection Station, Philadelphia, Pa.
**Practically free from foreign seeds and sand.

Where there is obvious difference in anatomical structure, such as in Acheen pepper—with many practically empty fruits—and Tillicherry pepper, even the crude method of using the graduate cylinder yields useful results.

Method II eliminates the element of chance in filling, though care must be taken that no air bubbles remain.

The data in the table, while limited, are very suggestive. They show that the results obtained by different collaborators with the same material agree fairly well. It is interesting to note that the difference existing in the specific gravity of whole Acheen and Aleppey peppers does not exist in the ground material Somewhat different results were expected, owing to the fact that Acheen pepper contains more shell and less starch than Aleppy. Eaton's results with different samples of Chinese mustard are worthy of special consideration. He points out that the sample with the highest specific gravity was adulterated with considerable dirt, sand and foreign seeds. The extent of usefulness of the apparatus is not definitely established.

## COMMENTS ON THE METHOD.

*E. O. Eaton.*—If the San Francisco samples are properly identified and apparatus is satisfactory, it would appear that the method would be of value as a means of identification only if samples are clean and free from foreign material.

*C. S. Brinton.*—I am not sure of the value of this determination until enough data has been obtained to show the maximum, average and minimum results on each product.

### COMMENTS ON THE APPARATUS.

*E. O. Eaton.*—The apparatus does not appear to hold kerosene well as it creeps out from ground-glass connections. A lead ring was used to weight it down. A non-greased buret (100 cc.) was used, the ground-glass connections being lightly water sealed.

*C. K. Glycart.*—It was noted that leakage resulted unless the ground-glass surfaces of the pycnometer were held firmly in place. It is suggested that a suitable clamp be devised, especially to insure the same adjustment of the volume.

*C. S. Brinton.*—I have measured one of our cream bottles and believe that the centrifuge and these wide-neck bottles would help considerably in getting more accurate results with many products as coriander, caraway, coffee, etc., which are rough and have crevices where air can be entangled. These cream bottles hold about 45 cc. and have a neck about 10 mm. inside diameter. They will easily hold 20 grams of most material. I should like to have tried this modification of your method but time did not permit.

### RECOMMENDATION.

It is recommended that the study of volume weight determinations be continued with the assistance of collaborators.

## MICROSUBLIMATION OF PLANT PRODUCTS.

By Arno Viehoever (Bureau of Chemistry, Washington, D. C.), *Associate Referee.*

The sublimation experiments[1] reported at the 1920 meeting, were continued with the collaboration of Ruth G. Capen and Joseph F. Clevenger. The apparatus consisted of a small beaker-like container with a small cup (approximately 1×1 cm.) extending from the bottom. The cup, containing the plant material, is heated in a cottonseed oil bath to the desired temperature. A small glass is placed over the opening and serves as a receiver for the sublimate. Some experiments were also made with an apparatus somewhat similar to that of Eder[2] in which vacuum and cooling were applied. Different plant products were tested with the results indicated below:

### *Artemisia cina and A. neo-mexicana.*

The flowerheads of these plants were heated in the sublimation apparatus. Santonin was obtained in a characteristic crystalline form and further identified in the following manner:

[1] *J. Assoc. Official Agr. Chemists*, 1921, 4: 414.
[2] Über die Mikrosublimation von Alkaloiden im luftverdünnten Raum. *Schweiz. Wochschr., Chem., Pharm.*, 1913, 51: 228-31, 241-5, 253-6.

*Identification of Santonin—*

(1) *Reaction with alcoholic furfural solution.*—The sublimate was dissolved in a very small amount of alcohol, to which 1 or 2 drops of alcoholic furfural solution and 1 to 2 cc. of sulfuric acid were added. Upon evaporation of the alcohol and especially after heating, the liquid is colored distinctly purplish red to carmine red; the color changes to bluish violet and deep blue, and finally shows a black precipitate. Alcoholic furfural alone with sulfuric acid, upon standing gives a pink or reddish color.

(2) *Precipitation with hydriodic acid.*—Hydriodic acid containing free iodine is added to an alcoholic solution of santonin. An olive green or greenish brown precipitate is formed, which shows characteristic crystallization, especially after the excess of the hydriodic acid is removed by decanting. Other reagents, such as ferrocyanic acid, ferricyanic acid and cobalticyanic acid did not give satisfactory results[1]. Both Capen and Clevenger obtained the crystals of santonin hydroperiodide, $(C_{15}H_{18}O_3)_2I_2HI$.

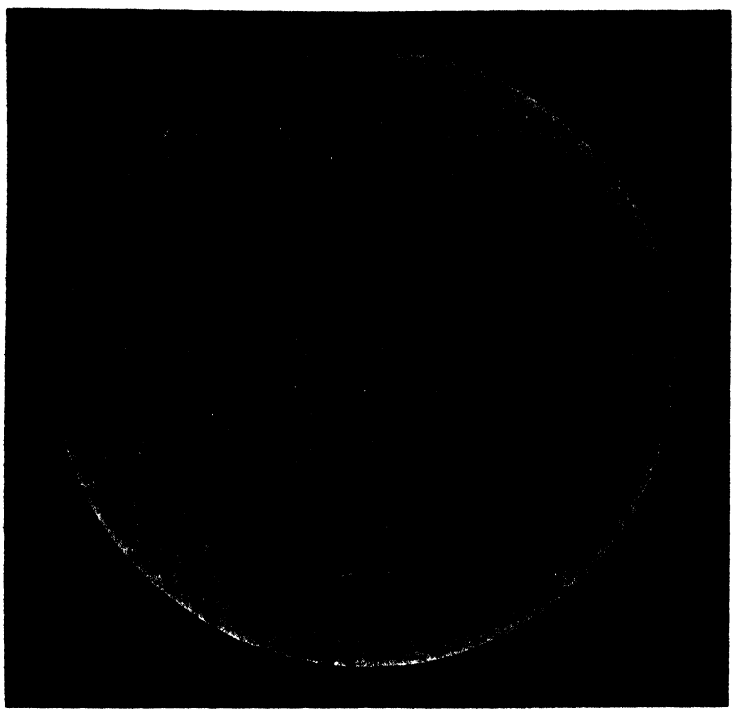


FIG. 1—×110
CRYSTALS OF SANTONIN HYDROPERIODIDE.

[1]Wedekind, E. Beiträge zur Kenntnis des Santonins. *Arch Pharm.*, 1906, 244: 623.

*Ilex cassine Michx.*

The fruits and seeds, upon microsublimation, yielded no caffeine. The leaves yielded caffeine which showed the characteristics of caffeine crystals.

*Identification of Caffeine—*

When treated with gold chloride solution, the crystals yielded yellow needles arranged in characteristic cluster form. They were, however, decomposed by water and alcohol. Very satisfactory crystals were obtained by the author and his collaborators upon addition of mercuric bichloride solution[1]. The change is especially characteristic if the bichloride solution is added directly to the sublimate and the transformation of crystals is observed under the microscope.

*Piper cubeba and Piper ribesioides.*

The sublimation of *Piper cubeba* var. *rinoe katoentjor* and *Piper ribesioides*, carried out with the collaboration of Capen, yielded interesting results. In both cases an oily sublimate was obtained; that of *Piper ribesioides* showed no crystals, even after treatment with ether, while that of *Piper cubeba* showed distinct needle-like crystals, which formed especially well after treatment with ether.

*Identification of Cubebin—*

Attempts to obtain diagnostic crystals by the addition of benzoylchloride to the cubebin sublimate have not been successful.

The blood-red color reactions with concentrated sulfuric acid is most characteristic. In the presence of traces only, a red tint was observed. Clevenger proved that a mixture of 50 parts of sulfuric acid with 50 parts of phosphoric acid was preferable to concentrated acid in the examination of the cubebs as well as of the sublimate.

*Hydrastis canadensis—Hydrastine.*

The sublimation of *Hydrastis canadensis* and the substitute *Jeffersonia diphilla* was carried out with the collaboration of Clevenger. In the case of *Jeffersonia* no crystalline sublimate was obtained, whereas goldenseal (*Hydrastis canadensis*) readily yielded numerous colorless crystalline structures.

## RECOMMENDATION.

It is recommended—

That the study of the microsublimation of plant products be continued with the assistance of collaborators.

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[1] Stevenson, C. H. Microchemical Tests for Alkaloids, 1921, Plates I and VIII.

# IDENTIFICATION OF CRUDE DRUG SUBSTITUTES.

By ARNO VIEHOEVER (Bureau of Chemistry, Washington, D. C.), *Associate Referee.*

In the report for 1920 the associate referee pointed out the characteristics useful in the identification of Spanish digitalis and Egyptian henbane. The association approved the recommendation that the method used be studied by collaborators. The distinguishing characteristics were checked by other workers and the following statements were submitted:

### SPANISH DIGITALIS.

*E. N. Gathercoal.*—There is no question but that your characteristics distinguishing Spanish from official digitalis are very well described. I can offer no suggestions for improvements whatsoever.

*E. E. Stanford.*—In regard to the characteristics of the two species of digitalis, my observation corresponds with yours. I believe, however, the margin of the Spanish digitalis is serrate, or biserrate, at least in some leaves. This characteristic has apparently, by inadvertence, been omitted from the printed description. As to the color of the drug, I recall very plainly the yellowish green character of the commercial samples which we had in the laboratory.

*C. W. Ballard.*—Margin of the *Digitalis thapsi* L. irregularly and minutely serrate dentate. In powdered materials the heads are apt to break from the glandular hairs. This fact might lead to confusion in the examination of powdered materials.

After receiving the reports of collaborators the method of identification[1] was amplified as follows:

*Digitalis purpurea* L.

*Stems.*—Usually present in small amounts or lacking in the drug.

*Digitalis thapsi* L.

*Leaves.*—Margin unequally serrate; veins less prominent.
*Stems.*—Usually present in large amounts in the drug.

### EGYPTIAN HENBANE

*E. N. Gathercoal.*—Regarding the Egyptian henbane, compared with the official drug, I have had very little opportunity of study. All of the samples of Egyptian henbane that I have met with consist of stems with no leaves or a small quantity of much broken leaves. I have never seen a whole leaf. Therefore, characteristics of the whole leaf probably would be of very little value to the examiner. However, your description of the microscopic distinction is good.

*E. E. Stanford.*—In regard to the two specimens of henbane, the sample submitted in my previous experience of Egyptian henbane does not enable me to corroborate or not to corroborate some of the characteristics you attribute to that species. The samples sent us, and almost all the other samples I have seen, consisted almost entirely of stems. Only small fragments of leaves were present. Likewise the capsules and the fragments of flowers were too much broken to enable me to check up their com-

[1] *J. Assoc. Official Agr. Chemists*, 1921, 4: 410.

parative length and prolongation. The light color of all this material which I have seen differentiates it sharply in the powder from the much greener, or grayish green shade of the powdered official henbane. This character or color and the microscopic evidence of a composition almost entirely of lignified stem tissues seem to me to be as valuable indications of the identity of powdered Egyptian henbane as the characters of the hairs. If one had to deal with a mixture of the two species, in which a relatively small proportion of Egyptian henbane was present, or with a sample of ground official henbane stems, the characteristics of the hairs which you enumerate would be correspondingly more valuable.

*C. W. Ballard.*—The distinguishing characteristics are very well stated. No comments are offered.

The method[1] of identification has been slightly amplified as follows:

*Hyoscyamus niger* L.

*Stems.*—Usually present in small amounts in the drug.

*Hyoscyamus muticus* L.

*Leaves.*—Usually much broken.
*Stems.*—Usually predominating in the drug.

FALSE CUBEBS.

The differentiation of cubebs and their substitutes was taken up as new work. The following table of distinguishing characteristics was submitted to the collaborators:

TABLE 1.

*Distinguishing characteristics of cubebs*.*

| FRUIT | *Piper cubeba* var. *rinoe katoentjor* | *Piper cubeba* var. *rinoe badak* | *Piper ribesioides.* |
|---|---|---|---|
| Shape | Nearly globular | Nearly globular | Nearly globular |
| Size | 3–6 mm. diam. | 3–6 mm. diam. | 5–8 mm. diam. |
| Color | Dark brown to grayish black | Grayish | Light gray to dark brown. |
| Thecaphore (fruit stem) | 5–7 mm. long | 5 mm. long | Up to 13 mm. long. |
| Stone cells | Inner layer radially elongated | Scattered through tissue of epicarp | Inner layer mostly isodiametric |
| Treated with concentrated sulfuric acid | Red to crimson | Deep brown | Brownish |
| Odor of ether extract | Normal | Mace-like | Somewhat turpentine-like |

*Compiled from literature and personal observations.

The following comments were received:

[1] *J. Assoc. Official Agr. Chemists*, 1921, 4: 411.

*E. N. Gathercoal.*—There is no question that the fruit of *Piper ribesioides* is generally larger, with a longer thecaphore, lighter in color, with isodiametric stone cells, a somewhat turpentine-like odor and responds with a brownish color when treated with concentrated sulfuric acid. We were not able to find any of the globular heads that were larger than 7 mm. in diameter and in a few instances the thecaphore measured more than 13 mm. long.

I was unable to distinguish *Piper cubeba* var. *rinoe badak* from *Piper cubeba* var. *rinoe katoentjor* except by the sulfuric acid test.

Of the 71 fruits, 15 turned brown instead of crimson red with concentrated sulfuric acid. Of these 3 were gray brown or grayish tan, 5 reddish brown and 7 dark brown. From your sample of the mixed varieties, of 4 selected light gray fruits, 3 reacted crimson and 1 brown with concentrated sulfuric acid. Of 4 selected dark brown fruits, 1 was brown with concentrated sulfuric acid and 3 were crimson.

Regarding the color of these varieties: We found in an examination of 2.890 grams of the mixed samples that there were 0.135 gram of stem, 0.690 gram formed by 48 very immature fruits and 71 fairly well formed fruits weighing 2.150 grams. These 71 fruits, by a careful examination of their color in a good light against a white background, were divided as follows:

| Color | No. of Fruits | Color | No. of Fruits |
|---|---|---|---|
| Blackish | 1 | Red-brown | 20 |
| Dark brown | 28 | Tan-brown | 8 |
| Dark brown with gray spots | 6 | Light gray or gray-tan | 3 |
| Gray-brown | 3 | | |

I could find no distinction between the two varieties in the character or arrangement of the stone cells in the tissues of the pericarp.

*C. W. Ballard.*—Stone cells appear scattered through the mesocarp of the *Piper cubeba* (*rinoe badak*) and the oil glands or cells appear much darker in color than in the other varieties. This difference may be due to the long boiling preliminary to sectioning but all samples were boiled the same length of time.

*Heber W. Youngken.*—I was able to verify most of the distinguishing characteristics on the list you submitted. I find, however, working on the material you sent me, the following characteristics which are at variance with the observations of your department. The color of the fruits of *Piper ribesioides* varies from light gray to light brown to dark brown. The thecaphore of *Piper cubeba* var. *rinoe katoentjor* showed a range in length from 5-9 mm.; *Piper cubeba* var. *rinoe badak* was 5-11 mm. long. * * *. All of these observations were checked by each of my advanced pharmacognosy students and verified.

The modified method of identification is as follows:

TABLE 2.

*Cubebs and substitutes*.*

| FRUIT | *Piper cubeba* var. *rinoe katoentjor* | *Piper cubeba* var. *rinoe badak* | *Piper ribesioides* |
|---|---|---|---|
| MACROSCOPIC CHARACTERS | | | |
| Shape | Nearly globular | Nearly globular | Nearly globular |
| Size | 3–6 mm. diam. | 3–6 mm. diam. | 5–8 mm. diam. |
| Color | Dark brown to grayish black | Grayish | Light gray to dark brown |
| Thecaphore (fruit stem) | 5–9 mm. long | About 5 mm. long | Up to 13 mm. long |
| MICROSCOPIC CHARACTERS | | | |
| Stone cells | Inner layer radially elongated | Scattered through tissue of epicarp | Inner layer radially isodiametric. |
| CHEMICAL CHARACTERS | | | |
| Treated with concentrated sulfuric acid | Red to crimson | Deep brown | Brownish |
| Odor of ether extract | Normal | Mace-like | Somewhat turpentine-like |
| Sublimate | White needle-like crystals | Oily mass, no crystals | Oily mass, no crystals. |

*Compiled from literature and personal observations.

## RECOMMENDATIONS.

It is recommended—

(1) That the modified method for the macroscopic and microscopic identification of *Digitalis thapsi* L. (Spanish digitalis), a recent substitute for *Digitalis purpurea* L., be adopted as a tentative method.

(2) That the modified method for the macroscopic and microscopic identification of *Hyoscyamus muticus* L. (Egyptian henbane), a substitute for *Hyoscyamus niger* L. be adopted as a tentative method.

(3) That the method for the macroscopic, microscopic and microchemical identification of cubebs (*Piper cubeba* var. *rinoe katoentjor* and its substitutes, *Piper cubeba* var. *rinoe badak* and *Piper ribesioides* Wall., be adopted as a tentative method.

# REPORT ON ALKALOIDS.

By A. R. Bliss, Jr.[1] (Emory University School of Medicine, Emory University, Ga.), *Associate Referee.*

The work on alkaloids for 1920-1921 involved (a) a study of the volumetric check on the weight of strychnine found by the assay for strychnine in tablets; (b) a study of a similar check in the assay for strychnine in liquids; (c) a study of the associate referee's method for the separation of quinine and strychnine[2]; (d) a study of the method for the assay of physostigma and its preparations[3]; (e) a study of a modified United States Pharmacopœia method for the assay of fluidextract of hyoscyamus[4]; and (f) a comparative study of the volumetric and gravimetric methods for the assay of ipecac and its preparations.

Carefully prepared samples and detailed instructions for the estimations were sent to F. W. Heyl, Upjohn Co., Kalamazoo, Mich.; E. M. Bailey, Connecticut Agricultural Experiment Station, New Haven, Conn.; E. C. Merrill, United Drug Co., Boston Mass.,; H. B. Mead, U. S. Food and Drug Inspection Laboratory, New York, N. Y.; G. E. Ewe, H. K. Mulford Co., Philadelphia, Pa.; Hugo H. Schaefer, Columbia University, College of Pharmacy, New York, N. Y.; L. Schwartz, U. S. Food and Drug Inspection Laboratory, New York, N. Y.; and M. F. Brown and W. H. York, Emory University Medical School, Emory University, Ga. Albin Stikarsfsky carried out the actual work on the samples submitted to E. C. Merrill, and R. E. Andrews carried out the work on the samples submitted to E. M. Bailey.

## ASSAY FOR STRYCHNINE IN TABLETS.

Carefully prepared tablet triturates, each containing 0.00043 gram of strychnine (standardized), were submitted to collaborators for assay by the following method:

Place 25 tablets in a 200 cc. Squibb separator and moisten with 8 cc. of water. Add 1 cc. of stronger ammonia water. Agitate with 25 cc. of chloroform and allow the mixture to stand until separation is complete. Draw off the chloroform into a second separator and repeat the agitation twice with 25 cc. portions of chloroform. Wash the combined chloroformic fractions with 10 cc. of distilled water and allow the mixture to stand 15 minutes. Introduce a pledget of absorbent cotton into the stem of the separator and run off the chloroform into a tared dish. (Do not allow the wash water to enter the orifice of the stop-cock.) Add 10 cc. of chloroform to the contents of the separator, and when the water has entirely risen to the surface run this chloroform also into the tared dish. Wash the outer and inner surfaces of the stem of the separator with a little chloroform, adding this also to the contents of the tared dish. Evaporate the chloroformic solution on a steam bath, using a fan or blower. Remove the dish

[1] Presented by A. G. Murray.
[2] *J. Official Agr. Chemists*, 1921, 4: 416.
[3] *Ibid.*, 418.
[4] *Ibid.*, 419.

from the bath as the last portions of chloroform evaporate, to avoid decrepitation. Dry at 100°C. to a constant weight and weigh as strychnine. (The U. S. P. factor for strychnine to strychnine sulfate is 1.2815.) Check the weight of the strychnine by dissolving the residue in neutral alcohol, adding an excess of 0.1N sulfuric acid, and titrating back with 0.02N potassium hydroxide, using methyl red as the indicator. (1 cc. of 0.1N sulfuric acid is equivalent to 0.0334 gram of strychnine or 0.0428 gram of strychnine sulfate.)

TABLE 1.

*Volumetric check in the results of assays for strychnine in tablets*.*

| ANALYST | GRAVIMETRIC METHOD | VOLUMETRIC CHECK |
|---|---|---|
| | *gram* | *gram* |
| G. E. Ewe† | 0.0107<br>0.0104 | 0.0133<br>0.0133 |
| A. Stikarsfsky‡ | 0.0127<br>0.0126<br>0.0128 | 0.01282<br>0.01216<br>0.01250 |
| M. F. Brown | 0.0108<br>0.0106<br>0.0103 | 0.0105<br>0.0104<br>0.0107 |
| W. H. York | 0.0106<br>0.0104<br>loss | 0.0109<br>0.0106<br>..... |
| A. R. Bliss, Jr. | 0.0107<br>0.0105<br>0.0104 | 0.0110<br>0.0103<br>0.0109 |
| Average..... | 0.01103 | 0.01149 |
| Theoretical..... | 0.01075 | 0.01075 |

* 25 tablets used.

† For some unaccountable reason the volumetric method gave results in excess of the gravimetric method, but in view of the very small quantity of alkaloid worked upon and the very small amount of standard acid consumed, the results have value as checks.

‡ Both methods seem to give results which are almost identical.

*Comments.*—Although the average gravimetric result is 0.01103 as compared to the theoretical quantity 0.01075, and the average volumetric result obtained by the same collaborators is 0.01149 as compared to the theoretical quantity 0.01075, when the small quantities of alkaloids worked with and the fact that the above averages are very much closer to the theoretical when the results of the second series listed in Table 1 are eliminated, are taken into consideration, it will be readily seen that both the method and the volumetric check are quite satisfactory.

## ASSAY FOR STRYCHNINE IN LIQUIDS.

(In the absence of other alkaloids.)

A carefully prepared elixir containing 0.0648 gram of strychnine (standardized) in 50 cc. was submitted to collaborators for assay by the following method:

Place about 50 cc. of the sample, accurately measured or weighed, into an evaporating dish and evaporate off the alcohol. Transfer to a 250 cc. Squibb separator. Add an excess of ammonia water and 25 cc. of chloroform. Agitate thoroughly and allow the mixture to stand until separation is complete. (Proceed as under the assay for strychnine in tablets, page 564.)

TABLE 2.

*Results of assays for strychnine in liquids.*

| ANALYST | GRAVIMETRIC METHOD | VOLUMETRIC CHECK |
|---|---|---|
| | *gram* | *gram* |
| G. E. Ewe. | 0.0668<br>0.0665 | 0.0671<br>0.0680 |
| A. Stikarsfsky* | 0.0636<br>0.0585<br>0.0581 | 0.0548<br>0.0542<br>0.0542 |
| F. W. Heyl† | 0.0616<br>0.0622<br>0.0581 | 0.05768<br>0.05010<br>0.05010 |
| M. F. Brown. | 0.0638<br>0.0635<br>lost | 0.0598<br>0.0636<br>..... |
| W. H. York. | 0.0637<br>0.0599<br>lost | 0.0632<br>0.0635<br>..... |
| A. R. Bliss, Jr. | 0.0650<br>0.0645<br>0.0639 | 0.0644<br>0.0642<br>0.0632 |
| Average..... | 0.06284 | 0.05994 |
| Theoretical..... | 0.0648 | 0.0648 |

* The volumetric determination seems to give invariably lower results. The drying to constant weight at 100°C. is rather prolonging the assay. To my mind the volumetric estimation, if reliable, is quicker and less troublesome.

† Oils troublesome.

*Comments.*—The results obtained by six collaborators, as shown in Table 2, indicate that both the method and the volumetric check are quite satisfactory.

## METHOD FOR SEPARATING QUININE AND STRYCHNINE.

A carefully prepared elixir containing 0.7776 gram of quinine (standardized) and 0.0258 gram of strychnine (standardized) in 50 cc. was used. The method submitted has been published[1].

TABLE 3.

*Results of separation of quinine and strychnine.*

| ANALYST | QUININE | STRYCHNINE |
|---|---|---|
| | *gram* | *gram* |
| G. E. Ewe. | 0.7875<br>0.7774 | 0.0215<br>0.0200 |
| A. Stikarsfsky* | 0.5428<br>0.5490<br>0.54325 | 0.02809<br>0.02788<br>0.02774 |
| F. W. Heyl† | 0.7878<br>0.7762<br>lost | 0.0255<br>0.0249<br>0.0252 |
| R. E. Andrews‡ | 0.7695<br>0.7690 | 0.0222<br>0.0245 |
| M. F. Brown | 0.7765<br>0.7772 | 0.0251<br>0.0246 |
| W. H. York | 0.7768<br>0.7699 | 0.0219<br>0.0242 |
| Average..... | 0.7232 | 0.02452 |
| Theoretical..... | 0.7776 | 0.0258 |

* Some resinous material remaining behind in gravimetric alkaloidal residue increases its weight, thus raising the amount of total alkaloids. During the extraction of quinine with ether flocculent resinous flakes separate and stick to the walls of funnel.
† Not enough ether to remove oils.
‡ Strychnine residue gave positive tests for quinine.

*Comments.*—The results obtained by six collaborators (not including the results obtained in a long series of experiments by the associate referee), as found in Table 3, indicate that this method is quite satisfactory and gives much more accurate results

[1] *J. Asso. Official Agr. Chemist*, 1921, 4: 416.

than the oxalate method[1], the tartrate method[2] or the ferrocyanide method as modified by Simmonds[3].

## ASSAY OF PHYSOSTIGMA AND ITS PREPARATIONS.

A manufacturer's extract of physostigma said to contain from 1.7 to 2.3 per cent of alkaloids was used.

The methods submitted for the assay of physostigma, fluidextract of physostigma (for alkaloids) and the tincture of physostigma (for alkaloids) have been published[4].

TABLE 4.

*Results of assays of extract of physostigma.*

| ANALYST | U. S. P. METHOD | SUBMITTED METHOD |
|---|---|---|
| | *per cent* | *per cent* |
| A. Stikarsfsky* | 1.775 | 1.635 |
| | 1.782 | 1.643 |
| | 1.787 | 1.651 |
| M. F. Brown | 1.776 | 1.8460 |
| | 1.784 | 1.8645 |
| | 1.785 | 1.8649 |
| W. H. York | 1.782 | 1.8527 |
| | 1.788 | 1.8682 |
| | 1.777 | 1.8478 |
| A. R. Bliss, Jr. | 1.789 | 1.8578 |
| | 1.786 | 1.8605 |
| | 1.782 | 1.8608 |
| Average..... | 1.782 | 1.80518 |

* The alkaloidal residue seems to be rather dark for titration by this method (submitted method), still it works well with methyl red.

*Comments.*—The results obtained by Ewe[5] and the associate referee and his collaborators, as shown in Table 4, indicate that the U. S. P. method gives much lower results (doubtless due to partial decomposition of the alkaloid and to incomplete extraction) than those obtained with the method recommended, which has proved quite satisfactory.

## ASSAY OF FLUIDEXTRACT OF HYOSCYAMUS.

A fluidextract very carefully prepared according to the method of the United States Pharmacopœia from a standard drug was submitted

[1] A. H. Allen, Commercial Organic Analysis, 4th ed. 1912, 6: 461.
[2] *Ibid.*, 462.
[3] *Analyst*, 1914, 39: 81.
[4] *J. Assoc. Official Agr. Chemists*, 1921, 4: 418.
[5] *J. Assoc. Official Agr. Chemists*, 1921, 4: 419.

to the collaborators to be assayed by the method given in the United States Pharmacopœia[1] with the following changes as suggested by H. C. Fuller, Institute of Industrial Research, Washington, D. C.:

Proceed as directed under "Fluidextractum Belladonnæ Radicis"[2], first line of the assay, modifying the process there by using 25 mils of the fluidextract of hyoscyamus in place of 10 mils of fluidextract of belladonna root, and adding *at least 30 mils of distilled water and 5 to 10 mils of stronger ammonia water*, and before titrating treating the residue twice with 5 mils of ether and evaporating to dryness each time.

TABLE 5.

*Results of assays of fluidextract of hyoscyamus.*

| ANALYST | U. S. P. METHOD | MODIFIED METHOD |
|---|---|---|
| | *gram* | *gram* |
| G. E. Ewe* | 0.0423 | 0.0477 |
| | 0.0423 | 0.0477 |
| A. Stikarsfsky† | 0.0466 | 0.0420 |
| | 0.0489 | 0.0396 |
| | 0.0494 | 0.0396 |
| M. F. Brown. | 0.0465 | 0.0520 |
| | 0.0458 | 0.0512 |
| | 0.0456 | 0.0515 |
| W. H. York. | 0.0452 | 0.0507 |
| | 0.0460 | 0.0518 |
| | 0.0455 | 0.0518 |
| A. R. Bliss, Jr. | 0.0450 | 0.0505 |
| | 0.0459 | 0.0515 |
| | 0.0452 | 0.0506 |
| Average..... | 0.0457 | 0.0484 |

* There is no doubt that the extra ammonia is desirable; it is the regular practice in this laboratory.
† The modified method gives invariably lower results. This is in my estimation due to the use of too much stronger ammonia water (10 mils). All evaporation was done on a water bath (not steam bath) until about 2 mils remained. These were evaporated in moderately hot water (not boiling). A too alkaline medium seems to hasten hydrolysis.

*Comments.*—The results of the modification of the U. S. P. method for the assay of fluidextract of hyoscyamus, obtained by five collaborators (Table 5), indicate that the recommended method is quite satisfactory.

[1] U. S. Pharmacopœia, IX, 1916: 187.
[2] *Ibid.*, 178.

## ASSAY OF IPECAC AND ITS PREPARATIONS.

*Method for emetine bismuth iodide.*

Weigh accurately about 0.5 gram of the salt into a glass-stoppered flask. Add 10 cc. of water and 3 cc. of ammonia water, shake and allow to stand 10 minutes. Add 50 cc. of ether, shake 10 minutes and then every 10 minutes during 2 hours. Decant 25 cc. of the ethereal layer into a 25 cc. graduated flask. Filter this through a pledget of cotton into a small tared beaker. Wash the flask and the filter with ether and add the washings to the contents of the tared beaker. Allow the ether to evaporate spontaneously, dry at 100°C. and weigh as anhydrous emetine. Multiply this weight by 2. The result is the weight of the total alkaloid in the sample.

Take up the alkaloidal residue with an excess of 0.02N sulfuric acid and titrate the excess with 0.02N sodium hydroxide, using cochineal as the indicator. Each cc. of 0.02N sulfuric acid consumed is equivalent to 0.0048 gram of anhydrous emetine.

It is impossible to present tabulated results as but two collaborators reported. However, the following data submitted by Ewe is important and interesting:

## NOTES ON PREPARATION OF EMETINE.

The method outlined by DuMez[1] consisted of precipitating an acidified aqueous solution of emetine hydrochloride with Dragendorff's reagent[2], collecting and washing the precipitate with water and drying it in the air at a temperature below 50°C. When so prepared the compound has an ugly brick-red color. But if the product is made by precipitating an acidified aqueous solution of emetine hydrochloride with Dragendorff's reagent, then warming the mixture slightly until the maximum bright red color consistent with no alteration of the emetine alkaloid is attained, a more beautiful and thoroughly combined product is obtained. The amount of heat required to effect the combination must be carefully controlled by experiment in order to prevent alteration of the emetine alkaloid which is sensitive to heat under the conditions of manufacture of this compound. The difference in results of alkaloidal assays of this compound by gravimetric and volumetric methods is a measure of the heat control; the volumetric method gives results only for unaltered alkaloid whereas the gravimetric method includes both altered and unaltered alkaloid. Therefore a properly made compound should give results which check closely by both methods.

Table 6 shows the results of assays of a number of samples of emetine bismuth iodide from various sources, by both volumetric and gravimetric methods.

Regarding the proper precautions to be taken in the assay method: All analyses, the results of which are given in Table 6, were made by placing about 0.3 gram samples in a separator, adding ether, decomposing with ammonia water, shaking out the alkaloid and collecting the ether extractions in a tared flask, recovering the ether and drying the residue of emetine alkaloid at 60°C., in the case of the gravimetric method. In the case of the volumetric method, the ether extractions were evaporated spontaneously in a beaker and the recovered alkaloid titrated as usual. It is essential that the ipecac alkaloids be protected from overheating. This can be accomplished by either evaporating the collected ether extractions spontaneously or, in order to save time, evaporating the collected ether extracts to small volume on the steam bath in a manner which prevents the steam from impinging on the flask anywhere except where it is in contact with the ether extracts and then finally evaporating the balance of the ether spontaneously. The proper heating of the flask can be accomplished by means of a steam

[1] *Philippine J. Sci.*, 1915, 10: 73.
[2] *Z. Analyt. Chem.*, 1866, 5: 407.

bath covered with removable rings, only the smallest ring being removed and the flask being placed directly over the small opening.

TABLE 6.

*Results of assays of anhydrous emetine alkaloid.*

| SAMPLE NO. | GRAVIMETRIC METHOD | VOLUMETRIC METHOD |
|---|---|---|
| | *per cent* | *per cent* |
| 1 | 26.99 | 21.3 |
| 2 | 22.85 | 17.94 |
| 3 | 27.75 | 19.84 |
| 4 | 27.4 | 14.63 |
| 5 | 28.2 | 28.2 |
| 6 | 24.8 | 24.8 |
| 7 | 27.2 | 25.13 |
| 8 | 26.0 | 23.15 |
| 9 | 25.02 | 23.35 |
| 10 | 29.8 | 23.4 |
| 11 | 24.82 | 24.6 |
| 12 | 19.84 | 19.3 |
| 13 | 27.0 | 21.5 |
| 14 | 22.5 | 14.1 |
| 15 | 22.8 | 17.6 |
| 16 | 32.7 | 31.5 |
| 17 | 22.5 | 22.1 |
| 18 | 28.2 | 28.2 |

It has been my experience that the sensitiveness of the ipecac alkaloids to heat is real but overrated. For instance, I made some experiments in 1914 along this line which I reported at the annual meeting of the American Pharmaceutical Association[1] that year as follows: "If the alkaloids of ipecac obtained during the assay process are allowed to remain on the steam bath after the ethereal layer has evaporated, darkening and disintegration of the alkaloids result. Of three experiments in which the alkaloids were kept at water-bath temperature for five minutes after the ethereal solvent had evaporated, 6.7%, 6% and 3% of the total amount of alkaloids present was lost". I also reported[2] later some experiments which I made regarding the effect of heat on emetine hydrochloride, which are also of interest along these lines.

Manufacturers are continually reminded of the sensitive nature of the ipecac alkaloids by the low alkaloidal yields in preparations of ipecac made by use of heat. The preparation which suffers most is the solid extract. The low yields in the case of the fluidextract are due primarily to incomplete initial extractions. It has been fairly well established that very little alkaloid remains in the extra percolate after it has been concentrated with heat to a small volume preparatory to adding it to the initial percolate. All of this evidence indicates the sensitiveness of the ipecac alkaloids and justifies the step in the assay of evaporating the ether extracts spontaneously or with the proper precautions against overheating as outlined previously.

It is my opinion that the difference in results obtained by gravimetric and volumetric methods with ipecac preparations is primarily due to alteration of the alkaloids (chiefly cephaeline) during the process of manufacture of the preparation; secondly, to the inclusion of non-alkaloidal matter in the alkaloidal residue; and thirdly, to alteration of alkaloids by the heat used during the assay. My statement that cephaeline

[1] *J. Am. Pharm. Assoc.*, 1914, 3: 1681.
[2] *Am. J. Pharm.*, 1919, 91: 275.

appears to be the offender is based on my experience in the manufacture of cephaeline alkaloid, which is extremely difficult to produce with the high titratable value shown by emetine.

## RECOMMENDATIONS.

It is recommended—

(1) That the method for the assay for strychnine in tablets, including the volumetric check, be adopted as an official method.

(2) That the method for the assay for strychnine in liquids (in the absence of other alkaloids), including the volumetric check, be adopted as an official method.

(3) That the associate referee's method for the separation of quinine and strychnine be adopted as an official method.

(4) That the method for the assay of physostigma and its preparations, as presented by G. W. Ewe, be adopted as an official method.

(5) That the method for the assay of fluidextract of hyoscyamus (which is a simple modification of the U. S. P. method) be adopted as an official method.

(6) That the study of the gravimetric and the volumetric methods for the assay of ipecac and its preparations be continued.

(7) That the assay of belladonna liniment by the method prescribed by the United States Pharmacopœia for *fluidextractum belladonnæ radicis*[1], as suggested by G. W. Ewe, be subjected to collaborative study with a view to its adoption as an official method.

NOTE.—The following experiment reported by Ewe indicates the absence of any effect of camphor on the assay: 10 cc. portions of a fluidextract of belladonna which assayed 0.758 (duplicate 0.726) gram alkaloid per 100 cc. were placed in separators, 0.5 gram portions of camphor added and the resulting solution assayed by the U. S. P. method for fluidextract belladonna. 0.740 gram alkaloid per 100 cc. was recovered.

(8) That the method for the assay of belladonna ointment, submitted by G. W. Ewe, which appears below, be subjected to collaborative study with a view to its adoption as an official method.

Place about 30 grams of belladonna ointment in an 8 ounce centrifuge bottle; add 150 cc. of a mixture of one volume of chloroform and two volumes of ether, followed by 10 cc. of ammonia water. Shake the bottle vigorously until all the fats are dissolved. Then shake on a mechanical shaker for four hours and let settle. Pour off the clear ethereal layer into a separator. Wash the residue in the centrifuge bottle with small portions of the ether-chloroform mixture; shake, let settle and pour off the clear ethereal layer. Collect the washings in a beaker, evaporate to small volume and wash the ether-chloroform extracts contained in the separator into the mixture. Extract the alkaloids by shaking out repeatedly with weak sulfuric acid and proceed as directed by the U. S. Pharmacopœia in the assay process for fluidextract belladonna root[1]. The assay can be hastened by centrifuging wherever instructions are given to let the mixture stand until it settles.

[1] U. S. Pharmacopœia, IX, 1916, 178.

NOTE.—This method is extremely accurate as shown by the following experimental data reported by Ewe: Approximately 3 gram samples, accurately weighed, of an assayed solid extract of belladonna leaves were placed in an 8-ounce centrifuge bottle, and the bottle placed in a beaker of warm water until the extract was softened. 1.6 cc. of dilute alcohol was added and worked into the extract with a thin glass rod. 10 grams of melted hydrous wool fat were poured into the bottle and mixed well. Then 18 grams of melted benzinated lard were poured in and also mixed in well with the rod. The rod was removed, wiped with filter paper and the filter paper also placed in the bottle. 150 cc. of chloroform-ether mixture were added, and the assay was proceeded with as described above. The following results are typical:

| PLAIN SOLID EXTRACT | | SOLID EXTRACT IN FORM OF OINTMENT | |
|---|---|---|---|
| | *per cent* | | *per cent* |
| Assay No. 1 | 2.023 | Assay No. 1 | 2.033 |
| Assay No. 2 | 1.946 | Assay No. 2 | 1.993 |
| Average | 1.984 | Average | 2.013 |

(9) That work be done on methods for assaying the U. S. P. ointment of stramonium.

(10) That work be done on methods for the determination of atropine, morphine, codeine and heroine in tablets.

## REPORT ON METHODS OF ANALYSIS OF MORPHINE, CODEINE AND HEROINE (DIACETYLMORPHINE).

By C. K. GLYCART (U. S. Food and Drug Inspection Station, Transportation Building, Chicago, Ill.), *Associate Referee.*

In accordance with the recommendation approved by the association at the last meeting, samples of morphine, codeine and diacetylmorphine of uniform composition were sent with directions for examination to the collaborators. The qualitative tests and quantitative methods submitted were substantially as reported in the preliminary work[1].

The following results were obtained by the collaborators—William Rabak, U. S. Food and Drug Inspection Station, Minneapolis, Minn.; H. McCausland, The Abbott Laboratories, Chicago, Ill.; and E. O. Eaton, United States Food and Drug Inspection Station, San Francisco, Calif.

### COMMENTS.

*E. O. Eaton.*—These methods all appear to me to be workable and I hope give good results. In the determination of heroine in heroine tablets it would appear that if morphine were present from the decomposition of the tablet some of it would be extracted by the method proposed and estimated as heroine.

*William Rabak.*—The figures represent results obtained by the direct titration of extracted alkaloids with 0.02N sulfuric acid solution without the use of 0.02N alkali. The methods which you submitted I found to be excellent in every detail.

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*J. Assoc. Official Agr. Chemists*, 1921, 5: 150.

*Results of analysis of alkaloids of opium.*

| SAMPLE NO | DRUG | RABAK | MCCAUSLAND | GLYCART | EATON |
|---|---|---|---|---|---|
| | | *per cent* | *per cent* | *per cent* | *per cent* |
| 1 | Morphine hydrochloride, U. S. P.... | 98.64<br>98.06 | 97.70<br>97.89* | 98.34<br>97.80 | 102.40<br>101.90 |
| 2 | Codeine sulfate, U. S. P..... | 103.84<br>103.23<br>..... | 104.40<br>104.20<br>..... | 104.90<br>104.90<br>104.10 | 104.10<br>104.10<br>..... |
| 3 | Diacetylmorphine hydrochloride, U. S. P..... | 98.14<br>98.14 | 100.34<br>99.95 | 99.82<br>99.73 | 100.70<br>100.70 |
| 4 | Powdered morphine sulfate tablets.. | 24.28<br>24.28 | 24.47<br>24.02 | 24.83<br>24.61 | 25.60<br>25.60 |
| 5 | Powdered codeine phosphate tablets. | 49.13<br>48.84 | 50.76<br>50.00 | 50.10<br>49.94 | 51.40<br>51.09 |
| 6 | Powdered diacetylmorphine tablets. | 12.63<br>12.54 | 13.35<br>13.28 | 13.06<br>12.89 | 13.20<br>13.30 |

* Check obtained by determination of chloride content.

*H. McCausland.*—

*Morphine:* The alkaloidal residue was treated with 2 cc. of methyl alcohol (commercial sample of highest purity), covered with a watch glass and heated, excess 0.02N sulfuric acid added, diluted with 50 cc. of water and titrated back with 0.02N sodium hydroxide.

*Codeine:* The residue was dissolved in a similar manner and titrated directly with 0.02N sulfuric acid.

*Heroine:* The residue was dissolved in a similar manner and titrated directly with 0.02N sulfuric acid.

*Codeine and heroine:* Used 20, 15, 10, 10 and 5 cc. of chloroform. Final chloroform washings after washing filtered through 7 cm. filter paper wet with chloroform. Neutral to litmus.

A uniform standardization of 0.02N sulfuric acid is suggested by dissolving about 150 milligrams of anhydrous brucine in 2 cc. of methyl alcohol and titrating directly. Brucine should be dried at 100°C. M. P. should be 178°C.

For this work, the salts of the alkaloids were selected from the preparations which are believed to be the most stable. In general, the United States Pharmacopœia has no assay for the salts of the alkaloids of opium. Analysis of several samples, purchased on the market, showed a variable content of crystal water; consequently the results reported were not expected to give a working control of 100 per cent.

The work as a whole shows that the methods are more satisfactory than those used at the present time. The results obtained are quite satisfactory, and the following recommendations are therefore submitted·

RECOMMENDATIONS.

It is recommended—

(1) That the qualitative and quantitative methods for the examination of morphine, codeine and diacetylmorphine, submitted at the last meeting, be adopted by the association as tentative methods.

(2) That these methods be further studied with the view of making them official.

## REPORT ON LAXATIVE AND BITTER TONIC DRUGS.

By HENRY C. FULLER (Institute of Industrial Research, Washington, D. C.), *Associate Referee.*

The work undertaken involved—

(1) The evolution of a method for assaying the anthraquinone drugs—cascara, rhubarb, senna and buckthorn—and their fluid extracts.

(2) The evolution of a method for assaying aloes and the adaptation of the same to preparations in which the laxative and bitter properties are characterized by aloes and aloin.

Specifications were drawn up for a method for assaying the anthraquinone drugs and submitted to a list of collaborators with carefully selected samples of powdered rhubarb, powdered senna and U. S. P. fluidextracts prepared from the same drugs.

*Method I.—Assay of Rhubarb, Senna and Cascara.*

(Use 2 grams of rhubarb, 5 grams of senna and 5 grams of cascara.)

Place sample in an Erlenmeyer flask, add 200 cc. of dry chloroform, attach to a reflux condenser using a cork stopper covered with tin foil, boil 15 minutes and then cool. Filter into a separatory funnel, washing with several successive portions (3 probably enough) of 40 cc. each of chloroform. Preserve the drug and filter for later treatment.

Add 50 cc. of 5% sodium hydroxide to the chloroform in the separatory funnel, shake thoroughly, let settle and draw off the chloroform layer into a clean separatory funnel. Repeat the extraction with 5% sodium hydroxide twice, using the same amount as in the first extraction. Discard the chloroform, combine the alkali solutions, add 15 cc. of ether, shake and let settle. Run off the alkaline solution and discard the ether. Add a slight excess of hydrochloric acid to the alkaline solution and shake out three times with 50 cc. of chloroform. Combine the chloroform extractions, wash with water and filter the chloroform through cotton inserted in the stem of the separator, into a tared dish. Evaporate, cool, dry in desiccator and weigh. This is the weight of the free oxymethylanthraquinones.

The powdered drug on the filter is then returned to the Erlenmeyer flask for the next step.

Add 200 cc. of chloroform and 50 cc. of 25% sulfuric acid. Boil for 2½ hours under a reflux. At the end of that time, transfer the contents of the flask to a separatory funnel, washing out the flask with a little fresh chloroform into the separatory funnel. Discard the acid. Add 50 cc. of 10% sodium bisulfite solution, shake thoroughly, let settle and draw off the sodium bisulfite. Add 100 cc. of 1% hydrochloric acid,

*Results of collaborative work on Method I.*

| COLLABORATOR | ANTHRAQUINONES. | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | RHUBARB | | | SENNA | | | FLUIDEXTRACT RHUBARB | | | FLUIDEXTRACT SENNA | | |
| | Free | Combined | Total | Free | Combined | Total | Free | Combined | Total | Free | Combined | Total |
| | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* |
| C. K. Glycart, U. S. Food and Drug Inspection Station, Transportation Bldg., Chicago, Ill. | 0.145<br>0.170 | 2.83<br>2.87 | 2.975<br>3.04 | 0.07<br>... | 1.08<br>... | 1.95<br>... | 0.68<br>0.72 | 1.00<br>0.98 | 1.68<br>1.70 | 0.280<br>0.296 | 0.690<br>0.780 | 0.970<br>1.076 |
| F. W. Casey, Bureau of Internal Revenue, Washington, D. C. | 0.52 | 4.39 | 4.91 | 0.16 | 3.21 | 3.37 | 1.00 | 1.49 | 2.49 | 0.590 | 1.310 | 1.904 |
| W. H. Blome, Frederick Stearns & Co., Detroit, Mich. | 0.44 | 2.75 | 3.19 | 0.296 | 2.74 | 3.036 | 0.26 | 1.73 | 1.99 | 0.300 | 0.776 | 1.076 |
| F. S. Rose, W. F. Severa Co., Cedar Rapids, Iowa. | 0.21 | 3.99 | 4.20 | 0.225 | 2.87 | 3.09 | ... | ... | ... | ... | ... | ... |
| J. M. Monran, Institute of Industrial Research, Washington, D. C. | 2.05 | 2.05 | 4.10 | 0.25 | 2.24 | 2.49 | ... | ... | ... | ... | ... | ... |
| Average | 0.297* | 3.14 | 3.73 | 0.20 | 2.58 | 2.78 | 0.66 | 1.30 | 1.96 | 0.366 | 0.894 | 1.310 |

* With 5 cmt

shake, let settle and draw off the acid. Then wash with 100 cc. of water, let settle and filter the chloroform, through cotton inserted in the stem of the funnel, into a distilling flask. Recover a portion of the solvent, pour into a tared dish, evaporate, dry in desiccator and weigh. Report percentage of free oxymethylanthraquinones and combined oxymethylanthraquinones.

For the fluidextract take a 5 cc. sample, spread on 5 grams of pure sawdust and evaporate on a steam bath. Transfer the dry sawdust to an Erlenmeyer flask and proceed with the determinations precisely as in the case of the powdered drug.

## SUGGESTIONS OF COLLABORATORS.

(1) According to the directions a large quantity of chloroform is required. If practicable, it would seem desirable to reduce the quantity of chloroform.

(2) In the assay of the fluidextracts it was found that some of the dried sawdust together with the material could not be removed completely from the dish by washing with chloroform, whereas a little 25% acid readily aided in the transfer.

(3) The results of analysis of the samples appear to be very satisfactory and the method gives much promise. If it is found by other collaborators to be satisfactory, it will prove very valuable in the examination of the class of products for which it is intended.

## DISCUSSION.

The results obtained by the collaborators are very encouraging to one who has had previous experience as a referee in cooperative analytical work. The novelty of the method and the fact that most of the collaborators were unable to make more than a single run on the samples submitted would lead one to anticipate much wider divergencies from the average figures than are reported. The details of new methods always require considerable polishing, and this is best accomplished by studying the comments of the collaborators and embodying their suggestions in the directions that are finally submitted for adoption. In this work the summations of the total anthraquinone derivatives in their general relation to the results obtained on the individual drugs are especially encouraging because they correspond very well with data in the literature on the subject. The results indicate that, with proper manipulation, a chemist will be able to satisfy himself concerning the quality of the bitter laxative drugs of the anthraquinone type, and that he can go a long way in estimating the quantity present in a mixture. It is interesting to note that the incorporation of these drugs into fluidextracts results in a considerable loss of anthraquinone derivatives.

At the time this work was being conducted your referee instituted some experiments on a colorimetric method based upon the hydrolysis of the anthraglucosides and the subsequent extraction of the oxymethylanthraquinones by ether which had been tested at the laboratory of the Bureau of Internal Revenue. The method as used in this laboratory is as follows:

*Method II.—Colorimetric Valuation of Rhubarb, Cascara and other Anthraquinone Drugs.*

Heat 0.5–2 grams of the finely powdered drug for 15 minutes with 50 cc. of 5% sulfuric acid under a reflux condenser. After cooling, extract the mixture with successive quantitites of ether until that solvent remains colorless when treated with a trace of potassium hydroxide. Heat the separated aqueous liquid to expel dissolved ether, boil for 15 minutes longer and again extract with successive quantities of ether. When no more soluble matter can be removed, shake the combined ethereal extracts with 200 cc. of a 5% solution of potassium hydroxide, in successive portions, until the alkaline liquid is no longer red. Dilute the red solution to 500 cc. with distilled water and transfer an aliquot of 10 cc. or more (depending on the depth of color of the liquid in the flask) to a Nessler tube, dilute to 50 cc. with distilled water and match against an alkaline aloe-emodin solution of the strength of 1–1,000,000.

This procedure was modified by comparing the color with an alkaline solution of the anthraquinone principles extracted from a good average sample of the drug under consideration. This should always be done when estimating the quantity present in a liquid preparation.

As an example of how this works out, a residue representing the free and combined anthraquinones from 2 grams of cascara was dissolved in 10 cc. of 10 per cent potassium hydroxide and the solution was made up to 100 cc. in a volumetric flask. Each cc. represented practically 0.3 grain of cascara. It was found that 2 cc. of this solution diluted to 50 cc. in a Nessler tube matched 10 cc. of a solution of the same character obtained from a product under review. In other words, 1 ounce or 30 cc. contained about 1.8 grains of cascara as determined by the recognizable principles.

## ALOES AND ALOIN.

An investigation of aloes and aloin was conducted conjointly with the work on rhubarb, senna and cascara.

Aloin is a peculiar substance differing from the anthraquinone bodies, being comparatively insoluble in the organic solvents. The readiness with which the anthraquinone derivatives dissolve in chloroform facilitated the development of a method for determining them.

Aloin readily combines with bromine. It was thought at first that a method of identification and possibly of estimation might be worked out based on this property, and it was found that in the case of pure aloin the figures obtained were fairly satisfactory. But when aloin occurred in admixture with other drugs the results were not reliable.

Aloin in solution apparently disappears rapidly if the liquid is left open to the air for any length of time. Just what happens to the substance is not known with certainty, but it is a fact that a solution of aloin which may be noticeably bitter at a dilution of 1 to 2,000 will, in a month's time, if open to the air or if enclosed in a bottle with considerable air standing over the surface of the liquid, become so reduced in its bitter manifestation that it may show bitterness at a dilution no

greater than 1 to 15. The loss which aloin undergoes under these conditions is confirmed by the bromine test noted previously, but the reaction is not sufficiently reliable to render it useful in quantitative work.

A method for determining the comparative bitterness of aloin solutions and thereby obtaining an approximate idea of the quantity present and the probable therapeutic effect was worked out in this laboratory. This method, submitted with samples to the collaborators for study with the rhubarb and senna samples, is as follows:

*Method III.—Determination of Degree of Bitterness of Aloes Mixture.*

Measure carefully 5 cc. of the liquid and introduce into a 100 cc. graduated cylinder. Dilute to 100 cc. and mix. Designate as Solution No. 1.

Take 5 cc. of this mixture, carefully measured, introduce into a 100 cc. graduated cylinder, dilute to 100 cc. and mix. Designate as Solution No. 2.

Introduce 5–10 cc. of Solution No. 2 into the mouth, allowing it to reach the anterior portion, and note whether or not there is any bitter taste.

If bitter, take 5 cc. of Solution No. 2, dilute to 100 cc. and test for bitterness. If this mixture is not bitter, make up further mixtures using 5 cc. of Solution No. 2 and gradually diminish the quantity of water. Note the dilution at which the last sensation of bitterness is apparent and then, based on the volume of the original mixture, figure the degree of dilution necessary to bring about the loss of bitterness.

## DISCUSSION.

The reports from the collaborators on this test were not very satisfactory and as the length of time the samples stood before the tests were made is uncertain, no figures will be submitted.

Finding it inadvisable to experiment further with the use of bromine as a reacting agent for estimating aloin, the study turned to a survey of the decomposition products which were obtained when aloin was subjected to the action of boiling acids and alkalis. Without going into detail as to the results of the numerous experiments conducted, it was finally ascertained that when boiled with sulfuric acid aloin was hydrolyzed, and one of the products of decomposition reacted with a derivative of paranitraniline forming a product very insoluble in water, and which was produced in constant quantity, relative to the amount of aloin originally in solution. As a result of this discovery it was believed that the way was opened for the development of a quantitative method for estimating aloin.

A series of tests was conducted in order to work out the proper conditions for running the method. As a result a set of specifications was evolved, which will be submitted to the collaborative chemists for their study. The method can be adapted to any kind of pharmaceutical preparation featuring aloes or aloin. The two procedures detailed below are the first of their kind ever proposed along these lines.

*Method IV.—Aloin Assay.*

Weigh out 0.2 gram of aloin into a 200 cc. Erlenmeyer flask fitted with stopper and attached to a reflux. Add 100 cc. of 25% sulfuric acid and boil 1 hour under the reflux. Cool in an ice mixture, dilute if necessary and add a slight excess of concentrated sodium hydroxide solution followed by 50 cc. of diazotized paranitraniline solution (1). Add hydrochloric acid until precipitation is complete and heat on a steam bath until it agglomerates. Filter on a creased filter paper, wash with water and pour hot C. P. acetone onto the precipitate, collecting the filtrate in a tared dish and adding sufficient acetone to take up all of the diazotized precipitate. Evaporate the acetone and dry residue at not over 100°C. Report weight of product.

Aloins probably vary to some extent in their constitution or at least in their character as articles of commerce. 0.2 gram of good commercial aloin gives a precipitate with the diazotized paranitraniline reagent, amounting to about 0.4861 gram.

(1) Treat 7 grams of paranitraniline with 12 grams of concentrated hydrochloric acid and 50 cc. of water and boil until solution is effected. Cool. Add 3.7 grams of sodium nitrite in 15 cc. of water. Cool. Dilute to 500 cc.

*Method. V.—Estimation of Aloin in Liquid and Solid Medicines.*

For assaying aloes or mixtures containing aloes or aloin the procedure will depend somewhat on the composition of the preparation. For a pill, tablet or any other galenical in solid form, a carefully comminuted sample should be thoroughly extracted with 95% alcohol. The mixture of alcohol and sample may, if desired, be put directly into a graduated flask and made up to the mark with 95% alcohol. In the case of pills or tablets, take 100 units and have the final solution made up to 500 cc.

If the sample is a liquid take a portion of reasonable quantity, say 25–50 cc., and having ascertained the weight, make up to 500 cc. with alcohol.

Of the solution in the 500 cc. graduated (volumetric) flask take an aliquot of 100 cc. of clear liquor and allow the alcohol to evaporate spontaneously, but better in a vacuum in order to lessen the time of exposure to outside influences. The residue should be under 100 cc.

Take up with water and transfer to a 100 cc. volumetric flask. (If aloes are under investigation take 10 grams; introduce directly into 100 cc. volumetric flask and add 50 cc. of water.) When all the aloin is in solution proceed as follows: Add lead acetate solution until in excess. Then make up to volume with water. Filter off an aliquot of 50 cc., transfer to a 100 cc. volumetric flask, add potassium oxalate crystals or solution in sufficient quantity to get rid of the excess of lead and make up to the mark. Filter off a 50 cc. aliquot, transfer to hydrolyzing flask, add 100 cc. of 25% sulfuric acid and heat under a reflux for 1 hour. Cool, transfer to separator and shake out with chloroform to remove any anthraquinone derivatives. Draw off the acid liquor into an evaporating dish and remove the chloroform at the temperature of the water bath. Return the liquor to a beaker or Erlenmeyer flask, add an excess of sodium hydroxide and precipitate with diazotized paranitraniline precisely as described in the aloin assay. Finish determination in the same way.

In cases where even the approximate quantity of aloes is not known, it is suggested that the liquid, after removing the chloroform, be transferred to a volumetric flask of 500 cc. capacity and made up to the mark. Then aliquots of 50–100–250 cc. may be taken for treatment with the diazotized paranitraniline.

RECOMMENDATIONS.

It is recommended—

(1) That the gravimetric method evolved for assaying the anthraquinone drugs be given a more exhaustive study during the ensuing year.

(2) That conjointly with the study of the gravimetric assay the collaborative work be extended to the colorimetric determinations.

(3) That the method for estimating aloin be submitted to the association for study and criticism.

No report on the determination of calomel, mercuric chloride and mercuric iodide in tablets was made by the associate referee.

## REPORT ON ACETYLSALICYLIC ACID.

By Arthur E. Paul (U. S. Food and Drug Inspection Station, 411 Government Building, Cincinnati, Ohio), *Associate Referee.*

Methods for the determination of aspirin must produce the separation of the aspirin from other substances which may be present, or be of such nature that foreign substances will not interfere. However, it would seem that it is necessary, first, to establish satisfactory methods for the determination of the product in question, without any special reference to possible interferences.

In the process of the spontaneous decomposition of aspirin, there is formed not only salicylic acid but also free acetic acid, and it would, manifestly, be of considerable interest to be able to determine this decomposition product. However, no entirely satisfactory method has as yet been devised. The only published method is that by A. Nutter Smith[1]. This method is correct in principle but somewhat cumbersome and unwieldly of execution. Copies of the method were submitted to collaborators for consideration with a view to making such changes or modifications as would result in a satisfactory procedure. No reports were received.

As it was believed impracticable to secure tablets of absolutely known composition and uniformity three samples in powder form, prepared from a well-known commercial make, were sent to collaborators. These were designated Sample No. 1, Sample No. 2 and Sample No. 3, and their composition was as follows:

| | | *per cent* |
|---|---|---|
| *Sample No. 1.*— | Commercial aspirin | 99.75 |
| | Salicylic acid | 0.25 |
| *Sample No. 2.*— | Commercial aspirin | 100.00 |
| *Sample No. 3.*— | Commercial aspirin | 80.00 |
| | Milk sugar | 20.00 |

[1] *Pharm. J.*, 1920, **105**: 90.

Collaborators were advised as to the general composition of the samples and that the preliminary extraction with chloroform might be omitted in working with Samples Nos. 1 and 2, but that Sample No. 3 would require the complete examination as prescribed.

## METHODS.

The following seven methods were submitted for study:

### *I.—Melting Point.*

If excipients are present, treat 0.2–0.3 gram with small portions of chloroform and filter into a beaker or dish. Evaporate the bulk of the chloroform on the steam bath and complete by spontaneous evaporation until thoroughly dry. Determine the melting point as directed in the U. S. Pharmacopœia[1].

### *II.—Qualitative test for free salicylic acid[2].*

Shake a 0.5 gram sample into a small Erlenmeyer flask with about 10 cc. of chloroform and filter. Evaporate, dissolve the dry residue in 10 cc. of cold water and filter. Add 1 drop of ferric chloride solution. (This should not produce more than a very faint violet color.)

### *III.—Quantitative method for free salicylic acid.*

(B. P. method, modified by P. N. Leach, Jr.[3] and by the associate referee.)

Prepare a standard salicylate solution as follows: Dissolve 0.1 gram of pure salicylic acid in 5 cc. of alcohol and dilute with distilled water to 1000 cc. Prepare also a 1% aqueous solution of iron alum (ferric ammonium sulfate).

In a Schreiner colorimetric tube dissolve a 0.2 gram sample in 1 cc. of alcohol and add 48 cc. of water. Filter if necessary. In a second colorimetric tube place 5 cc. of the standard salicylic acid solution, 1 cc. of alcohol, 1 drop of acetic acid, and dilute as above. Now add 1 cc. of the iron solution to each tube. Let stand 5 minutes and compare the colors in the colorimeter.

### *IV.—Iodine method for total salicylates.*

Heat a 0.1 gram sample in a 200 cc. Erlenmeyer flask with 20 cc. of water and 1 gram of sodium carbonate on a steam bath for 15 minutes. Filter if necessary to remove talc. Dilute to 100 cc., heat nearly to boiling, then add slowly 25–40 cc. of strong (about 0.2N) iodine solution, and proceed as directed for sodium salicylate[4]. Multiply the weight of the precipitate by 0.4016 to obtain the total salicylic acid and deduct the free salicylic acid previously determined. The difference represents the combined salicylic acid. Multiply by 1.304 to obtain the weight of aspirin.

### *V.—Bromine method for total salicylates by Koppeschaar reagent.*

Prepare 0.1N bromine solution as described in the U. S. Pharmacopœia[5].

Saponify a 0.5 gram sample with 10 cc. of 2% sodium hydroxide solution by heating for 15 minutes on a steam bath. Dilute with water in a measuring flask to 500 cc. Transfer an aliquot portion of this solution, containing not less than 0.040 gram nor

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[1] U. S. Pharmacopœia, IX, 1916, 596.
[2] Allen's Commercial Organic Analysis, 4th ed., 504.
[3] *J. Ind. Eng. Chem.*, 1918, **10**: 288.
[4] *Assoc. Official Agr. Chemists, Methods*, 1920, 298.
[5] U. S. Pharmacopœia, IX, 1920, 558.

more than 0.050 gram of acetylsalicylic acid, to a 500 cc. glass-stoppered Erlenmeyer flask, add 30 cc. of the 0.1N bromine solution and 5 cc. of strong hydrochloric acid, and immediately insert the stopper. Shake repeatedly half an hour and allow to stand for 15 minutes. Remove the stopper just sufficiently to introduce quickly 5 cc. of 20% potassium iodide solution, taking care that no bromine vapors escape, and immediately stopper the flask. Shake thoroughly, remove the stopper and rinse it and the neck of the flask with a little distilled water so that the washings may flow into the flask. Titrate with 0.1N sodium thiosulfate solution, using starch as the indicator.

Each cc. of 0.1N bromine corresponds to 0.002301 gram of salicylic acid, or to 0.003001 gram of acetylsalicylic acid.

### *VI.—Double titration method for acetylsalicylic acid*[1].

If excipients are present, treat about 0.3 gram, accurately weighed, with small portions of chloroform; filter into a beaker and wash until completely extracted. Evaporate the bulk of the chloroform on the steam bath, finishing with the aid of an electric fan without heat. Dissolve the chloroform-soluble residue in 10 cc. of alcohol.

If excipients are absent, dissolve the sample directly in the alcohol.

Titrate immediately with 0.1N alkali, using phenolphthalein as an indicator. (This titration should be made rapidly, and the first persistent pink color used as the end point, since any slight excess of alkali has a tendency to hydrolize the ester quickly.)

Add a volume of 0.1N alkali equal to that used in the first titration and then an excess of 5 cc. Heat on the water bath for 15 minutes. Titrate back with 0.1N acid.

If the product is pure, the total amount of alkali consumed will be twice that of the first titration. Each cc. of 0.1N alkali consumed in the two titrations is equivalent to 0.009 gram of acetylsalicylic acid.

### *VII.—Free acetic acid.*

The method submitted was that given by Smith in his paper, "A Method for the Determination of Free Acetic Acid Present in Acetyl-Salicylic Acid (Aspirin)"[2].

The collaborators reporting were the following: C. K. Glycart, A. W. Hanson and H. O. Moraw, U. S. Food and Drug Inspection Station, Chicago, Ill.; and William Rabak, U. S. Food and Drug Inspection Station, Minneapolis, Minn.

## COMMENTS BY COLLABORATORS.

*A. W. Hanson.*—No trouble was experienced with any of these methods for aspirin. The bromine titration method is believed to be the quickest and most satisfactory for total salicylates where a number of determinations are to be made.

*C. K. Glycart.*—The melting points of the samples of aspirin appear to be lower than found in the literature. The qualitative and the quantitative methods readily show the presence of free salicylic acid. The iodine and bromine methods and particularly the double titration method promise to be satisfactory in the valuation of aspirin preparations.

*H. O. Moraw.*—I: In the sample containing excipients, the residue from the chloroform extract was not sufficiently dry to pour into the capillary tube without previous drying. Dried it between filter papers. III: The chemical name for iron alum should be given either alone or in parentheses. In the second paragraph, the directions for diluting would be clearer if changed to read "and 43 cc. of water" or "and dilute to same volume as above". (This refers to the tube containing the standard.)

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[1] *Pharm. Ztg.*, 1913, **58**: 26.
[2] *Pharm. J.*, 1920, **105**: 90.

*Results of determinations by collaborators.*

I.—Melting point, results expressed as °C.
II.—Qualitative test for free salicylic acid.
III.—Quantitative method for free salicylic acid, results expressed as per cent.
IV.—Iodine method for total salicylates, results expressed as per cent.
V.—Bromine method for total salicylates by Koppeschaar reagent, results expressed as per cent.
VI.—Double titration method for acetylsalicylic acid, results expressed as per cent.

| | Sample No. 1* | | | | Sample No. 2† | | | | Sample No. 3‡ | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Glycart | Hanson | Rabak | Moraw | Glycart | Hanson | Rabak | Moraw | Glycart | Hanson | Rabak | Moraw |
| I | 130.5<br>131.0 | 135<br>..... | 134<br>135 | 128<br>..... | 129.8<br>130.5 | 134<br>..... | 132<br>134.5 | 128.7<br>..... | 129.8<br>130.5 | 130<br>131 | 132<br>130.5 | 130.5<br>..... |
| II | Violet | Violet | Strong | Distinct violet | Faint violet | Faint violet | Faint | Very faint violet | Trace | Faint violet | Faint | Very faint violet |
| III | 0.38 | 0.45 | 0.35 | 0.41 | 0.09 | 0.15 | 0.06 | 0.11 | 0.06 | 0.15 | 0.05 | 0.05 |
| IV | 97.80<br>97.09 | 98.0<br>98.0 | 97.40<br>97.66 | 98.86<br>97.55 | 98.13<br>98.84 | 99.2<br>99.2 | 97.66<br>97.80 | 99.3<br>99.2 | 78.55<br>78.03 | 80.0<br>79.5 | 77.71<br>77.97 | 79.78<br>79.26 |
| V | 96.97<br>99.02 | 98.7<br>99.0 | 96.33<br>96.33 | 99.39<br>99.39 | 98.34<br>98.00 | 99.3<br>98.7 | 98.43<br>97.83 | 99.4<br>100.5 | 77.58<br>77.85 | 79.9<br>80.3 | 79.22<br>78.62 | 82.2<br>81.9 |
| VI | 99.6<br>99.3 | 98.7<br>98.4 | 99.6<br>99.6 | 99.0<br>98.7 | 99.6<br>100.25 | 99.6<br>100.1 | 99.90<br>99.60 | 99.62<br>99.60 | 79.95<br>80.10 | 78.0<br>78.6 | 79.80<br>79.20 | 79.80<br>79.94 |

* Aspirin present, 99.75%; salicylic acid, 0.25%.
† Aspirin present, 100%.
‡ Aspirin present, 80%; milk sugar, 20%.

## DISCUSSION.

The comments by Moraw are quite valuable, since they indicate the points in the methods as submitted which are not entirely clear. The details suggested may be incorporated into the methods without constituting any real change therein, and it is thought that they will render the methods more easily understood by an analyst who undertakes to use them for the first time.

## CONCLUSION.

The results reported on the melting point vary considerably, but as a whole agree very well with those of Leach, who made a rather extensive study of the melting point of this substance. His conclusion is that purified acetylsalicylic acid melts at about 132°C. and that various commercial brands melt between 128° and 133°C. Sharp readings can hardly be expected since aspirin is a condensation product which readily undergoes decomposition, particularly at elevated temperatures, and becomes quite unstable at or near its melting point.

Reference is usually made in the literature to the melting point of aspirin, and since the results obtainable are sufficiently close to be of some value in the examination of samples, the official methods might well include details for this determination. This would seem particularly necessary since a deviation from a definite set of details would probably result in a still greater divergence in results.

The qualitative test for free salicylic acid, as might be expected, is entirely satisfactory.

The correct quantitative results for the samples submitted for free salicylic acid are not known as it was not possible to secure a sample which gave entirely negative tests. But it will be observed that the results obtained on Sample No. 1 (aspirin with 0.25% salicylic acid added), after deducting the free salicylic acid found in the aspirin used (Sample No. 2), are quite acceptable. The differences represent the percentages of added salicylic acid, according to the results reported by each collaborator, and are as follows:

| | *per cent* |
|---|---|
| C. K. Glycart | 0.29 |
| A. W. Hanson | 0.30 |
| William Rabak | 0.29 |
| H. O. Moraw | 0.30 |

(Actually added, 0.25 per cent.)

The results obtained in the determination of total salicylic acid by both the gravimetric and volumetric methods were quite satisfactory.

The double titration method is the simplest and quickest for evaluating a sample, and it is the most satisfactory method when working with tablets known to contain only pure aspirin and the ordinary excipients.

It must be remembered, however, that this method is not specifically for aspirin, and that it does not differentiate between this product and other similar substances. It is therefore necessary, in working on unknown samples, to supplement the double titration results by such determinations as are included in this report, which are more specific. It is believed that if all the methods given in this report are applied to a sample, the results will show quite satisfactorily the proportion of aspirin present, provided that the figures are consistent. It is not believed, however, that the methods are sufficient in all possible sorts of mixtures.

## RECOMMENDATIONS.

It is recommended—

(1) That the method for the determination of the melting point as given in this report be adopted by this association as a tentative method.

(2) That the qualitative test for free salicylic acid, as given in this report, be adopted as a tentative method.

(3) That the quantitative method for salicylic acid, substantially as given in this report but including the details suggested by H. O. Moraw, be made a tentative method, and that it be resubmitted to collaborators by next year's associate referee with a view to its adoption as an official method.

(4) That the iodine method for total salicylates, as given in this report, be made a tentative method, and that it be further tried out by next year's associate referee with a view to its final adoption as an official method.

(5) That the bromine method for total salicylates, as given in this report, be made a tentative method, and that it be further tried out by next year's associate referee with a view to its final adoption as an official method.

(6) That the double titration method for acetylsalicylic acid, as given in this report, be made a tentative method, and that it be further tried out by next year's associate referee with a view to its final adoption as an official method.

(7) That A. Nutter Smith's method for free acetic acid, and any other available methods for this determination, be submitted to collaborative study by next year's associate referee.

(8) That consideration be given to methods for the quantitative determination of combined acetic acid in acetylsalicylic acid.

(9) That the problem of determining aspirin in the presence of possible interfering substances be given consideration by next year's associate referee.

No report on methods for the examination of phenolphthalein was made by the associate referee.

# REPORT ON METHODS FOR THE DETERMINATION OF MONOBROMATED CAMPHOR IN TABLETS.

By C. D. WRIGHT (Bureau of Chemistry, Washington, D. C.), *Associate Referee.*

In accordance with the recommendations of last year, cooperative samples were prepared from two varieties of tablets purchased in the open market and distributed to eight collaborators, together with copies of two methods of determination essentially as published by W. O. Emery[1] and E. O. Eaton[2].

The methods follow:

*Method I.*

When an aqueous alcoholic solution of monobromated camphor is subjected to the action of sodium amalgam, on heating, among other changes the bromine is split off quantitatively in the form of its sodium salt, which may then be determined gravimetrically in the usual way.

REAGENT.

*Sodium Amalgam.*—To 100 grams of pure mercury which has been slightly warmed, contained in a small porcelain mortar, add about 1 gram of bright metallic sodium, cut in several pieces, by impaling the pieces successively on the point of a file and holding submerged in the mercury until the reaction, which is rather violent, is complete. Keep the resulting 1% amalgam in a tightly corked bottle.

DETERMINATION.

Count and weigh a suitable number of tablets to ascertain the average weight; reduce to a fine powder and keep tightly stoppered. Weigh out a portion corresponding to 0.1 to 0.2 gram of monobromated camphor, and transfer quantitatively with 20 cc. of alcohol and 10 cc. of water, to a small (100 cc.) round-bottomed flask, containing 15 grams of 1% sodium amalgam. Connect the flask, by means of a rubber stopper, with a vertical reflux. Heat the mixture over a wire gauze just to boiling for a period of not less than 30 minutes. After cooling slightly, wash out the condenser tube first with 5 cc. of alcohol, then with 5 cc. of water, receiving the washings in the flask below. Remove the flask to the steam bath and heat for another hour, or until the evolution of hydrogen has nearly or quite ceased. Toward the latter part of this operation, render the liquid about neutral with a few drops of acetic acid in order to further reduction. Transfer the contents of the flask to a separatory funnel, preferably of the Squibb type, withdrawing and washing the mercury in a second separatory funnel with at least two 50 cc. portions of water. Pass the several aqueous solutions quantitatively through a small filter, collecting the clear filtrate in a suitable beaker. Precipitate with silver nitrate after the addition of about 5 cc. of nitric acid, and proceed with the determination of the resulting silver bromide in the usual gravimetric way, employing, if available, a Gooch crucible in the operation of filtering. The weight of silver bromide multiplied by the factor 1.23 will give the quantity of monobromated camphor originally present in the portion taken for analysis. A control should be run on the amalgam in order to determine whether any correction is necessary for the presence of halogen in material quantity.

[1] *J. Ind. Eng. Chem.*, 1921, **11**: 756.
[2] *Ibid.*, 1922, **14**: 24.

*Method II.*

Monobromated camphor is decomposed in hot alcoholic potash solution by the addition of alcoholic silver nitrate solution and the resulting potassium bromide determined as silver bromide.

PREPARATION OF SAMPLE.

Determine the average weight in the usual manner and grind to a fine powder. Keep tightly stoppered.

DETERMINATIONS.

Weigh out in a small beaker an amount equivalent to about 0.2 gram of monobromated camphor, add 25 cc. of alcohol, warm on the steam bath and filter into a flask (preferably about 250 cc. and provided with a ground-in condenser), washing both beaker and filter with warm alcohol. Add 50 cc. U. S. P. normal alcoholic potassium hydroxide and 25 cc. of alcoholic silver nitrate (0.2 gram in 50 cc. of alcohol) and connect with a reflux condenser. Boil gently 1½ hours, adding at intervals through the condenser the remaining 25 cc. of the alcoholic silver nitrate solution. Cool, disconnect, and transfer the contents to a large evaporating dish. Dilute to 200 cc. and decant into a beaker, washing the sediment with water by decantation. Boil the solution 5 minutes with 1 gram of zinc dust to clarify; filter into another beaker, washing thoroughly with water, and add dilute nitric acid to decided acidity and aqueous silver nitrate solution to complete precipitation. When the silver bromide has agglutinated, filter on a weighed Gooch, wash with water and alcohol, dry at 100°C. and weigh. (The factor for monobromated camphor is 1.23.)

Four reports were received and the results are tabulated below:

*Results of determinations for monobromated camphor.*

| ANALYST | SAMPLE 1 | | SAMPLE 2 | |
|---|---|---|---|---|
| | Method 1 | Method II | Method I | Method II |
| | *per cent* | *per cent* | *per cent* | *per cent* |
| E. O. Eaton, U. S. Food and Drug Inspection Station, San Francisco, Calif. | 76.9 | 75.6 | 18.0 | .... |
| | 78.1 | 76.5 | 18.0 | .... |
| A. W. Hanson, U. S. Food and Drug Inspection Station, Chicago, Ill. | 75.9 | 75.0 | 17.0 | 17.3 |
| | 75.3 | 75.7 | 17.1 | 17.8 |
| W. F. Kunke, Bureau of Chemistry, Washington, D. C. | 75.0 | 73.2 | 17.0 | 18.7 |
| | 74.5 | 72.2 | 16.9 | 18.1 |
| | .... | 74.7 | .... | 17.7 |
| | .... | 74.4 | .... | .... |
| C. D. Wright. | 73.4 | 75.2 | 17.7 | 18.4 |
| | 74.7 | 76.7 | 17.7 | 16.5 |
| | .... | .... | .... | 17.0 |
| | .... | .... | .... | 16.3 |
| Average | 75.5 | 74.9 | 17.4 | 17.5 |

COMMENTS.

The results, while not remarkably concordant, are perhaps sufficiently representative to indicate the degree of variance to be expected in the

hands of different analysts. In the case of Sample 1, a difference of 1% is produced by an error of 1 to 2 milligrams in the silver bromide weighed; with Sample 2 an error of 8 milligrams is required to produce the same difference. Sample 2, however, contained vegetable extractives, and it is suggested that a final washing of the silver bromide with alcohol would be especially desirable in this case, and would do no harm in all cases as a precaution.

An evident omission in Method II is a caution to correct for the halogen present in reagents by a blank determination. This is very essential in view of the use of potassium hydroxide and of zinc dust, both of which commonly contain appreciable amounts of chloride. In this connection it may be stated that equally good results were obtained by Kunke and the writer by omitting the washing by decantation of the precipitated silver oxide in Method II, as well as the treatment with zinc dust, and instead, after diluting the alcoholic solution in the flask with water, filtering directly into a beaker and proceeding with the precipitation by silver nitrate. Whether this could be safely done in all cases is, however, a matter for further study.

### RECOMMENDATIONS.

It is recommended—

(1) That Methods I and II for the determination of monobromated camphor in tablets be adopted as tentative.

(2) That further study be made of Method II with a view to its possible simplification.

## REPORT ON METHODS FOR THE EXAMINATION OF PROCAINE (NOVOCAINE).

By Alfred W. Hanson (U. S. Food and Drug Inspection Station, Transportation Building, Chicago, Ill.), *Associate Referee.*

The method submitted last year for the examination of procaine was studied further, as well as the extraction and titration of the procaine base from an ammoniacal solution. Samples were sent to collaborators, and the results have been tabulated in an endeavor to determine the most satisfactory method. The results obtained from collaborators in 1920 showed that the bromide-bromate titration method gave good results on samples ranging from 0.02 gram to 0.20 gram.

### DESCRIPTION OF SAMPLES.

No. 1.—Contained 12.15 per cent of procaine, balance sodium chloride.

No. 2.—Contained 23.38 per cent of procaine, 0.28 per cent of adrenalin, balance sodium chloride.

No. 3.—Consisted of commercial procaine tablets, powdered. Each tablet was labeled as containing 0.02 gram of procaine and 0.00002 gram of adrenalin, balance sodium chloride. Amount of procaine, calculated 58.8 per cent.

No. 4.—Consisted of procaine.

The procaine used in the mixtures was the same as Sample No. 4. Sodium chloride was added to the first two samples as that is the substance used in commercial tablets.

The qualitative tests and the bromide-bromate quantitative method (designated Method I in this report), submitted to the collaborators, were essentially the same as given in last year's report[1].

*Qualitative methods.*

REAGENTS.

(a) *Mercuric potassium iodide (Mayer's reagent).*—Dissolve 1.3 grams of mercuric chloride in 60 cc. of water, add 5 grams of potassium iodide dissolved in 10 cc. of water and make to 100 cc.

(b) *Potassium permanganate solution.*—Dissolve 5 grams of potassium permanganate in water and make to 100 cc.

QUALITATIVE TESTS.

(1) Dissolve 0.1 gram of procaine in about 10 cc. of water. Add 2 cc. of potassium permanganate solution. Warm if necessary. Reduction occurs with evolution of gas having the odor of acetaldehyde. (Distinction from cocaine, which does not readily reduce potassium permanganate.)

(2) Dissolve about 5 mgs. of procaine in 3 cc. of water and add a few drops of mercuric potassium iodide (a). In the case of procaine, a white precipitate is formed which dissolves if a few cc. of dilute sulfuric acid (2%) are added. (The precipitates with mercuric potassium iodide formed with stobaine and cocaine are not readily soluble in dilute sulfuric acid.

(3) Dissolve about 0.1 gram of procaine in 2 cc. of water. Add 25 cc. of 0.1N sodium hydroxide. (A white precipitate is formed which dissolves in excess of sodium hydroxide when heated on a steam bath.) The alkali should be added from a buret. Heat the solution for 25 minutes on a steam bath. Upon cooling the solution and extracting with chloroform, no residue should be obtained upon evaporation of the chloroform. (Stovaine does not readily hydrolize and a residue would remain upon evaporation of chloroform, giving an alkaloidal reaction.)

(4) Dissolve 0.1 gram of novocaine (procaine) in 5 cc. of water. Add 2 drops of hydrochloric acid and 2 drops of sodium nitrite solution. Pour the mixture slowly into 10 cc. of a solution of 0.2 gram of betanaphthol in 10% sodium hydroxide solution. A scarlet red precipitate is formed.

(5) Add 5 drops of potassium permanganate solution to 0.1 gram of novocaine in 5 cc. of water and 3 drops of hydrochloric acid. The violet color disappears immediately (distinction from cocaine hydrochloride).

(6) Dissolve about 0.1 gram of procaine (novocaine) in 1 cc. of sulfuric acid. The solution remains colorless showing absence of organic impurities.

The following modifications may be noted:

In making the determination, it is advisable to run a control on 25 or

[1] *J. Assoc. Official Agr. Chemists*, 1921, 5: 163.

50 cc. of the potassium bromide-bromate solution alongside and under the same conditions as for unknown.

Results given under Method I modified were obtained by titration of the extracted procaine base by the potassium bromide-bromate method. The base was extracted from an ammoniacal solution according to Method II. The oily residue, consisting of the procaine base, was dissolved in 2 cc. of 95 per cent alcohol. A slight excess of 0.1N hydrochloric acid was added and the alcohol removed by evaporating almost to dryness on the steam bath. Method I[1] was followed from this point commencing with the addition of an excess of 25 cc. 0.1N sodium hydroxide and hydrolysis on the steam bath.

The proposed bromine titration method has the following advantages:

(1) Good end point.

(2) Very low 0.1N factor as compared with the 0.1N factor for acid titration, thus enabling the determination of smaller amounts of the compound.

(3) The titration by this method can be performed on procaine direct without first extracting the base as required by the other method. Procaine is sold in the form of a powder or compressed into tablets with salt. It has been found that chlorides do not interfere with this titration.

(4) The method is found to be rapid and accurate.

(5) The potassium bromide-bromate volumetric solution is stable.

(6) As cocaine and stovaine do not titrate by this method, it serves to differentiate these substances from procaine.

In the case of tablets, it may be advisable to take enough to make a representative sample and make up to a definite volume, using a volumetric flask. Aliquots of the clear solution can then be taken for the quantitative determinations. Insoluble substances like starch and talc can be removed in this manner.

### *II.—Gravimetric and Volumetric Method.*

#### EXTRACTION AND TITRATION.

Weigh an amount of the powder or take enough tablets to equal about 0.2 gram of procaine. Dissolve the sample in a few cc. of distilled water. Transfer the solution to a separatory funnel and add about 3 cc. of ammonia. Extract the ammoniacal solution 4 or 5 times with chloroform. Use 15 cc. of chloroform for the first extraction and 10 cc. for the other extractions. Keep the volume of the aqueous solution small. Filter and evaporate the chloroform extractions in a tared beaker. Evaporate the chloroform by means of an electric fan, preferably at room temperature. Avoid prolonged heating of the procaine base, as it appears to be slightly volatile at 100°C. Calculate the amount of procaine in the sample by multiplying the weight of extracted residue by the factor 1.1546. Take up the residue with a slight excess of 0.1N or 0.02N acid. Titrate back the excess of acid with 0.02N sodium hydroxide and methyl red indicator. The 0.1N factor for procaine hydrochloric is 0.027265.

The results of the determinations by the different methods using varying samples obtained by the associate referee; H. McCausland, 4753 Ravenswood Ave., Chicago, Ill., and J. H. Bornmann, 1625 Transportation Bldg., Chicago, Ill., collaborators, are shown in the following table:

[1] *J. Assoc. Official Agr. Chemists*, 1921, 5: 163.

*Results of determinations using different methods on different samples.*

| | Sample No. 1* | | | Sample No. 2† | | | Sample No. 3‡ | | | Sample No. 4¶ | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Bornmann | McCausland | Hanson | Bornmann | McCausland | Hanson | Bornmann | McCausland | Hanson | Bornmann | McCausland | Hanson |
| | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* |
| Method I | 11.5 | 11.98 | 11.6 | 22.7 | 23.02 | 23.00 | 63.4 | 62.60 | 63.20 | 98.5 | 96.45 | 99.0 |
| | 11.2 | 11.10 | 12.2 | 22.7 | 23.20 | 23.00 | 62.9 | 63.70 | 61.70 | 98.5 | 95.04 | 99.6 |
| | .... | 11.39 | 11.4 | .... | .... | .... | .... | .... | .... | .... | 97.61 | 99.2 |
| | .... | .... | .... | .... | .... | .... | .... | .... | .... | .... | 98.82 | 99.2 |
| Method I (modified) | 11.6 | .... | 11.2 | 22.4 | .... | 22.80 | 61.3 | .... | 62.79 | 98.9 | .... | 98.3 |
| | 11.5 | .... | 11.5 | 22.4 | .... | 21.80 | 60.9 | .... | 61.40 | 97.8 | .... | 98.7 |
| Method II (gravimetric) | 12.5 | 12.52 | 12.9 | 23.7 | 23.84 | 25.10 | 63.8 | 66.26 | 65.20 | 100.1 | 100.1 | 100.2 |
| | 13.0 | 12.10 | 12.7 | 24.4 | 23.16 | 24.60 | 65.6 | 65.58 | 66.60 | 101.8 | 100.4 | 100.7 |
| Method II (volumetric) | 10.9 | 12.53 | 11.1 | 20.5 | 23.83 | 23.04 | 57.5 | 65.66 | 63.60 | 93.3 | 100.1 | 96.3 |
| | 11.3 | 12.14 | 11.4 | 20.9 | 23.32 | 22.80 | 56.1 | 65.15 | 63.80 | 93.5 | 100.0 | 96.0 |

* Procaine present, 12%.
† Procaine present, 23.38%.
‡ Powdered commercial tablets; procaine declared, 58.8%.
¶ Procaine.

## COMMENTS ON THE QUALITATIVE TESTS.

*J. H. Bornmann:*

*Test No. 1.*—Procaine reduces permanganate almost instantaneously, with the formation of acetaldehyde, which may be recognized by odor.

*Test No. 2.*—Procaine yields a milky precipitate with Mayer's reagent, which dissolves in a few cc. of 2% sulfuric acid. Cocaine yields a curdy precipitate and stovaine a milky precipitate, neither of which dissolves in 2% sulfuric acid.

*Test No. 3.*—This test was tried upon procaine, cocaine and stovaine. In no case was a test obtained with Mayer's reagent on the residue from extraction. Contrary to the statement made in the outline, stovaine is hydrolized by this treatment.

NOTE BY REFEREE.—As originally written, the test was to be made on a 0.02 gram sample. This sample was found too small, and the directions were changed to 0.1 gram. Stovaine gives a positive reaction if present in this amount.

*Test No. 4.*—Procaine yields a dark red precipitate and a dark red solution. In the case of cocaine the precipitate is pale yellow, while the precipitate from stovaine is bright yellow. In each case, the precipitate eventually dissolves, giving a red solution in the case of procaine and yellow in the case of cocaine and stovaine.

## COMMENTS ON THE QUANTITATIVE METHOD.

*J. H. Bornmann.*—The results obtained by extraction and weighing appear to be too high, while those obtained by acid titration of the extracted base appear to be too low. The bromine titration method gives very good results considering the small amount of material used, and where there are no foreign substances present to react with the bromine, the determination is best made on the material direct without extraction.

*H. McCausland.*—The volumetric method using 0.02N sulfuric acid has been used by the collaborator for two years, except that he dissolved the final residue in 2 cc.of neutral alcohol, titrating directly with 0.02N sulfuric acid. If the amount exceeds 0.1 gram he prefers dissolving the base in an excess of 0.02N sulfuric acid and titrating back with 0.02N sodium hydroxide. He states that he was not satisfied with the results obtained by the bromide-bromate titration of procaine but, taken as a whole, the results are very good.

## DISCUSSION.

The results obtained by the different methods show that the bromide-bromate method is the most accurate. (See results of analysis.) Where small amounts of procaine are to be analyzed it is important to have a solution with a small 0.1N factor and the bromide-bromate method possesses this advantage. In titrating procaine base dissolved in alcohol with standard acid, using methyl red indicator, the end point is not very sharp.

## RECOMMENDATIONS.

It is recommended—

(1) That the qualitative tests and the bromide-bromate quantitative method presented at the last meeting of the association, with the modification of the quantitative method referred to herein, be adopted as a tentative method for the determination of procaine.

(2) That the method be studied during the next year with a view of making it official.

# PRELIMINARY REPORT ON METHODS FOR THE SEPARATION AND ESTIMATION OF THE PRINCIPAL CINCHONA ALKALOIDS.

By ELGAR O. EATON (Food and Drug Inspection Station, U. S. Appraiser's Stores, San Francisco, Calif.), *Associate Referee.*

It first appeared possible to separate quinine from the other cinchona alkaloids by the relative insolubility of its sulfate, as shown by Duncan[1]. Considerable experimenting was done, using as a basis its precipitation in a slightly ionized acid solution (dilute acetic acid) with excess sulfate ions (addition of sodium sulfate). Analysis of the sulfate precipitate by polariscopic methods showed that it contained other alkaloids, notably cinchonidine.

Gustav Mossler[2] pointed out this difficulty, but the writer verified it to his satisfaction. Mossler concluded that sulfate, chromate and oxalate formed double salts with quinine and cinchonidine and that no chemical separation of these two alkaloids on a scale suitable for assay work was possible at present. Experiments verified this and it was concluded, finally, that chemical separation of quinine and cinchonidine on a small scale is not feasible.

The use of solvents as a means of separation was abandoned after looking up the solubilities and trying a few experiments.

The idea of taking advantage of the methoxyl group in quinine as a means of separating it from cinchonidine does not appear practicable as a quantitative method.

After considerable work along these lines, experiments based upon the separation of quinine and cinchonidine from other cinchona alkaloids by their precipitation as tartrates (proposed by Koppeschaar, 1885) and their estimation in the combined precipitate by polariscopic methods as suggested by Hesse[3], Oudemanns[4], Lenz[5] and others appear to be most promising and to give fairly accurate results if all conditions are carefully controlled.

This experimental work was limited to quinine, cinchonidine and cinchonine.

## DETERMINATION.

Take sufficient sample to give approximately 0.3 gram of total alkaloids and dissolve in dilute sulfuric acid. Filter if necessary, make ammoniacal and extract with chloroform to exhaustion; evaporate; dry at 110°C.; and weigh. Dissolve in 50 cc. of reagent "N" (225 cc. of normal sulfuric acid diluted to 1000 cc.), heat on a steam bath 10 minutes and make just alkaline with dilute sodium hydroxide solution; then

[1] *Pharm. J.*, 1909, 82: 429.
[2] *Pharm. Monatsch.*, 1920, 1: 2-7, 17-22.
[3] Ann. Chem. (Liebig) 1875, 176: 203; 1876, 182: 128; 1880, 205: 217.
[4] *Ibid.*, 1876, 182: 33.
[5] Ztschr. Chem. 1888, 27: 549.

faintly acid, using dilute acetic and methyl red indicator. Add 25 cc. of saturated Rochelle salt solution (neutralizing just before using with dilute acetic acid and methyl red indicator); place in the ice-box, stirring occasionally for 2 hours. Filter and wash with a cold, half-saturated, neutralized Rochelle salt solution, using a small wash bottle and stirring precipitate on filter with a stirring rod to remove all aklaloids. Save combined filtrate and washings (Solution "A") for determination of cinchonine. (The tartrate precipitate will contain quinine and cinchonidine and is designated as Group I.) Decompose the precipitate with dilute sulfuric acid and transfer to a 250 cc. Squibb-type separatory funnel; wash the beaker and filter with dilute acid, shaking out with 5 cc. of chloroform to remove the methyl red. Wash the chloroform with 5 cc. of dilute acid, discard the chloroform and add the acid to the first separatory funnel. Make solution ammoniacal, shake out with four 20 cc. portions of chloroform, evaporate in a tared beaker containing a few grains of sharp sand, dry at 100°C. a few minutes, cool and weigh. Add 1 cc. of reagent "N" for each 0.015 gram of alkaloids, let stand 15 minutes, stir with policeman to complete solution and transfer to a polariscopic tube, filtering if necessary. (If a saccharimeter is used, reading to only -20°V., it will be necessary to use a 100 mm. tube. If an angular rotation instrument is available use a longer tube for greater accuracy.) Take the reading in angular degrees at 20°C., using sodium light or a bichromate filter with white light. If only a small amount of liquid is available an ordinary polariscopic tube can still be used by decreasing its capacity by inserting a straight thin-walled glass tube of 4 or 5 mm. internal diameter and about 5 mm. shorter than the polariscopic tube. Round its edges slightly in a flame and fix rigidly in the observation tube, using a portion of a 1-hole rubber stopper in the enlarged end of the tube and a bit of thin rubber tube at the smaller end. (These tubes can be readily filled by means of a small funnel held close to the inner edge of the polariscope tube at the smaller end, where the short inner tube leaves a small cup. The use of small-bored inner tubing for polariscopic use was first brought to my attention in an unpublished article by A. G. Murray.)

Take half of the total solution, calculated from the amount of reagent "N" used to dissolve Group I, and determine alkaloids by above shake-out method; evaporate and dry at 110°C. for 2 hours; cool and weigh. (It appears that the alkaloids read lower after drying at 110°C.)

Calculate the quinine and cinchonidine as follows:

$$\text{(A)}\ {}^{20}_{D} = \frac{10000 \times a}{L \times c} \text{ (specific rotation), in which}$$

a = observed degrees in angular rotation;
L = length of tube in mm.; and
c = grams of anhydrous alkaloids per 100 cc. as ascertained by shaking out and weighing after polarization.

Now substitute the value of (A) ${}^{20}_{D}$ in the formula[1]

$$100 \frac{\text{(A)}\ {}^{20}_{D} - 180}{277.4 - 180} = \text{Per cent of quinine in the total anhydrous alkaloids of Group I.}$$

The total anhydrous alkaloids, i. e. twice the weight of anhydrous alkaloid found minus the weight of quinine found equals the cinchonidine in Group I. Calculate the percentage of each in the original mixture from the weight of the sample used.

Make solution "A" ammoniacal, shake out with four 20 cc. portions of chloroform,

[1] Optical Rotation of Organic Substances. Landolt-Long, 1902, 500.

evaporate the solvent, dry at 110°C. for 2 hours and weigh. Dissolve in reagent "N" (1 cc. for each 0.015 gram of alkaloid), polarize, and calculate the specific rotation. In the absence of other cinchona alkaloids than quinine and cinchonidine, this should correspond reasonably well with that of cinchonine if present in an essential amount (+ 260), since quinine and cinchonidine are but slightly soluble in the menstruum used to precipitate Group I.

If quinine, cinchonidine or cinchonine occur alone, it can be readily estimated by simple extraction and determination of the specific rotation in acid solution under conditions outlined previously.

Quinine sulfate after recrystallization four times in the laboratory was found to give a specific rotation of -277.4. Specimens of cinchonidine sulfate and cinchonine sulfate, U. S. P. quality, showed respectively specific rotations of −180 and +260, all calculated to anhydrous alkaloids. This checks closely with the literature.

Solutions of these three alkaloidal salts were prepared to contain 0.015 gram of anhydrous alkaloids per cc. mixed in different proportions and assayed by the above methods with the following results:

*Recovery by proposed method.*

| MIXTURE | QUININE | | CINCHONIDINE | | CINCHONINE | |
|---|---|---|---|---|---|---|
| | Taken | Recovered | Taken | Recovered | Taken | Recovered |
| | *gram* | *per cent* | *gram* | *per cent* | *gram* | *per cent* |
| 1 | 0.225 | 93.3 | 0.075 | 102.0 | 0.075 | 108.0 |
| 2 | 0.120 | 97.0 | 0.150 | 94.0 | 0.045 | * |
| 3 | 0.225 | 94.0 | 0.060 | 90.0 | 0.045 | * |
| 4 | 0.150 | 90.0 | 0.150 | 105.0 | 0.150 | 101.0 |
| 5 | 0.180 | 90.0 | 0.120 | 107.0 | 0.045 | * |

* Not determined.

This method appears to be promising for the analysis of mixtures of these three alkaloids.

## RECOMMENDATION.

It is recommended that the method outlined in this report for the separation and estimation of the principal cinchona alkaloids and any other methods that may be available be studied by the association during the next year.

# THE DIFFERENTIATION OF JAPANESE AND AMERICAN PEPPERMINT OILS.

By ELGAR O. EATON (Food and Drug Inspection Station, U. S. Appraiser's Stores, San Francisco, Calif.), *Associate Referee.*

Large quantities of Japanese oils, which are derived from *Mentha arvensis*, are imported into this country. The United States Pharmacopœia recognizes peppermint oil for drug purposes as the volatile oil from *Mentha piperita* and Circular 136[1] recognizes the same for food purposes; consequently, it is obvious that from the standpoint of food and drug inspection a means of differentiating these oils is desirable. Much of this imported oil is dementholized, but its constants are still very close to the United States Pharmacopœia article.

Several color tests have been proposed for the identification of peppermint oil, but those given in the United States Pharmacopœia 1890[2] appear to be best. These tests have been combined and modified by the writer so as to give the maximum color in the minimum time.

*Modified Test.*

Add 5 drops of the oil to 1 cc. of glacial acetic acid in a small test tube and then 1 drop of concentrated nitric acid. Heat the mixture in the water bath to about 60°C., hold there for 1 or 2 minutes and note the color changes. A violet or bluish color develops in *Mentha piperita* oil in a few minutes when observed by transmitted light and a copper colored fluorescence by reflected light. Japanese oils usually show a straw color and sometimes a very faint blue color, but no copper fluorescence.

Eighteen American, one English and five Japanese peppermint oils were collected from various sources and subjected to this test. The American and English samples were obtained from Fritzsche Bros., New York City; A. M. Todd, Kalamazoo, Mich., and George Lueders & Co., San Francisco, Calif. Of the American oils, six were stated to have been produced in Michigan, three in Indiana, two in New Jersey and one in Oregon. The producing state was unknown or not given for the remaining American samples. Three of the Japanese samples were taken from direct importations at San Francisco and two were obtained from certain of the above dealers. The date of distillation of some of the oils was furnished by the dealers or distillers. Seven of the American oils were distilled from 1918 to 1920, one in 1905 and the two New Jersey oils were stated by Fritzsche Bros. to have been distilled in 1874. Some of the oils were single distilled and some were twice rectified.

All the American and English samples gave positive color reaction, while the Japanese oils were negative. In order to ascertain the effects

[1] U. S. Dept. Agr. Office of the Secretary Circ. 136: (1919) 16.
[2] U. S. Pharmacopœia, VII, 1890, 281.

of different sorts of storage upon the proposed test, portions of these different samples were put in colorless, partially filled, glass containers, some of which were left open and some of which were closed. Portions of some of the samples were held where direct sunlight could reach them and others were stored in diffused light. The various specimens were tested at intervals of several weeks to see whether the color reaction had been affected. After about two months it was noticed that the American oils held in open containers in direct sunlight gave a negative reaction. Also, one or two samples held in diffused light for six months gave nearly negative reactions. Attempts were made to treat the oils which had lost the property of giving the positive color test, so as to restore it. It was found that this could be done by distilling with steam and at the same time treating with nascent hydrogen, as described below.

*Regeneration Treatment.*

Place 5 cc. of oil in a volatile acid distilling apparatus[1] and add 10 cc. of 2.5N sulfuric acid and 1 gram of zinc (mercury amalgamated) or add 1 drop of platinic chloride solution. Distil with steam, collecting the distillate in a 4-ounce separatory funnel; draw off the water; filter the oil and test as before.

In every case the American oils which had lost the ability to give the reaction recovered that property on being treated. Two Japanese oils, similarly stored and subsequently distilled as above, still gave a negative test, showing only a very faint blue tint, which faded rapidly, with no copper fluorescence.

Experiments were tried to ascertain whether the change from a positive to a negative reacting oil might be accomplished by other agencies, such as freezing out part of the menthol, by steam distillation or by action of different catalytic agents, such as iron oxide, platinic chloride, platinum black and hydrochloric acid. None of these agents caused the oils to lose their positive reaction. Treatment with concentrated nitric acid or with chlorine was found to destroy the positive test. It was not learned what constituent or constituents of the oil are affected by storage with exposure to light and air, but it is thought to be an oxidation effect.

Organoleptic comparisons were made between some of the American oils in their original condition and the same oils after storing under such conditions that they had lost the power of giving a positive test, as well as the Japanese oils in their original condition. Five drops of 10 per cent alcoholic solution were placed on cubes of sugar, and the odor and taste were observed. Four observers agreed in general that the modified American oils and the Japanese oils tasted bitter and lacked the characteristic odor of the United States Pharmacopœia oil.

[1] *Assoc. Official Agr. Chemists, Methods*, 1920, 177.

The specific gravity and the index of refraction were determined on some of the original oils, as well as on some of the oils modified by light and air, with the following results:

| | ORIGIN | INDEX REFRACTION 20°C. | SPECIFIC GRAVITY 25°C./25°C. |
|---|---|---|---|
| Pure peppermint oil | Japan | 1.4580 | 0.892 |
| Peppermint oil (dementholized) | Japan | 1.4580 | 0.894 |
| Pure peppermint oil | Japan | 1.4580 | 0.894 |
| Peppermint oil (Japanese fraction) | Japan | 1.4576 | ..... |
| Oil peppermint, Natural—not U. S. P. | United States | 1.4595 | 0.8996 |
| Oil peppermint, Michigan pure single distilled | United States | 1.4595 | ..... |
| Oil peppermint, U. S. P. redistilled | United States | 1.4600 | ..... |
| Oil peppermint, pure natural—non-rectified | United States | 1.4600 | ..... |
| Oil peppermint | United States | 1.4599 | ..... |
| Oil peppermint | United States | 1.4616* | ..... |
| | | 1.4613* | ..... |

*After modification 1.4631.

## CONCLUSIONS.

It appears possible to distinguish American from Japanese peppermint oils by the above described procedure, regardless of age, rectification or manner of storage since distillation. The test, however, will not indicate the admixture of Japanese with American oils.

The index of refraction, as well as the specific gravity, is lower for Japanese oil than for the American.

The odor and taste of Japanese oil and of improperly stored American oils were not so pleasant as those of the United States Pharmacopœia article.

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